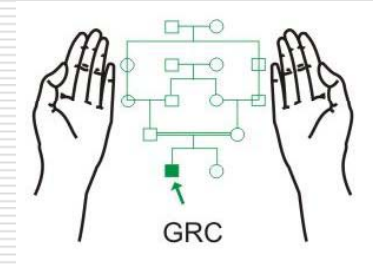


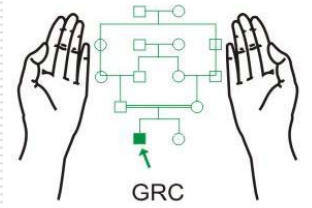
PCR Applications in Inherited Disorders

Maj Gen (R) Suhaib Ahmed, HI (M)
MBBS; MCPS; FCPS; PhD (London)

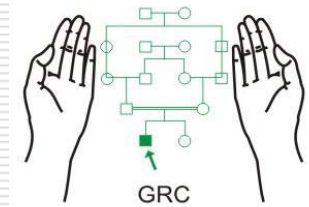
Genetics resource Centre (GRC)



www.grcpk.com



Genetic or Inherited Disorder?



Genetic Disorders

□ Chromosomal Defects

- Trisomies (Trisomy 21 Down's syndrome)
- Monosomies (45,X Turner syndrome)
- Deletions

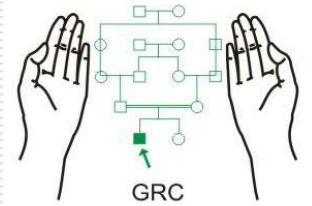
□ Single Gene Defects

- Autosomal
 - Recessive (Thalassaemia)
 - Dominant (Achondroplasia)
- X-linked (Haemophilia)

□ Polygenic Defects

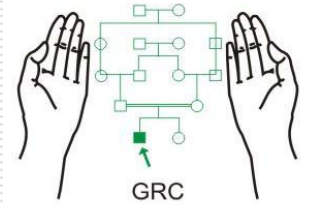
- Diabetes, IHD, Peptic Ulcer etc.
-

Diagnosis of Inherited Disorders

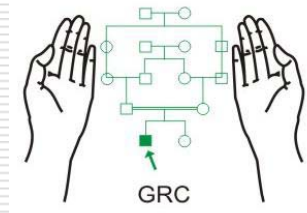


- Clinical presentation
 - Symptoms since birth
 - Family history
 - Siblings
 - Cousins
 - Other family members
 - Distant relatives
 - Consanguinity
-

Diagnosis of Inherited Disorders

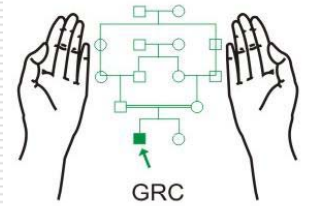


- Routine Tests
 - Biochemical Tests
 - Cytogenetics
 - Molecular Genetics**
 - Other related investigations
-



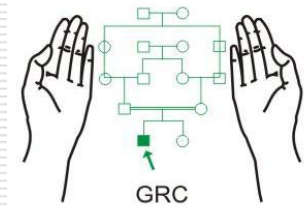
**Of the 5000 known Genetic Disorders
2500 can be diagnosed by Genetic Analysis**

Diagnosis of Inherited Disorders



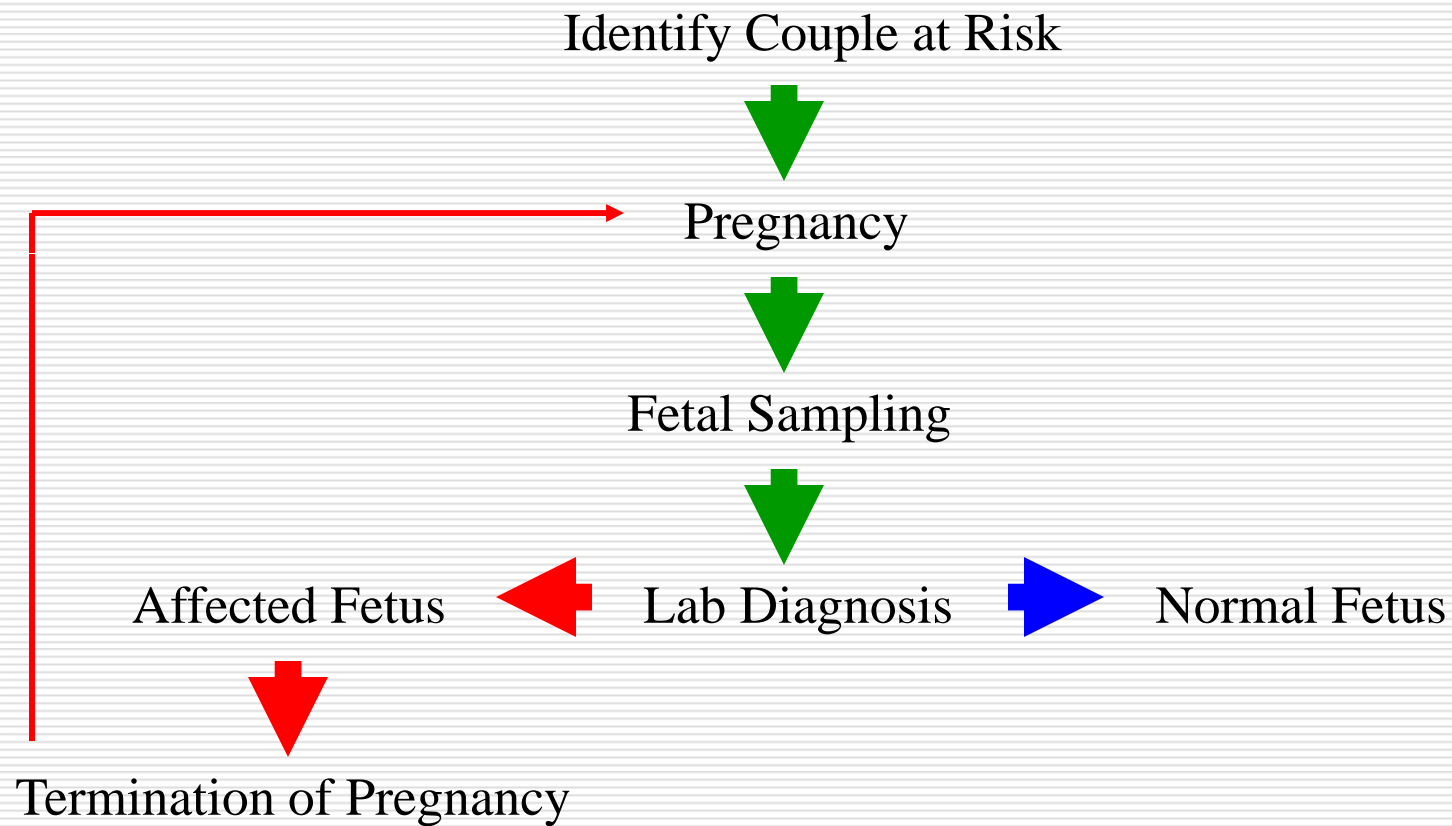
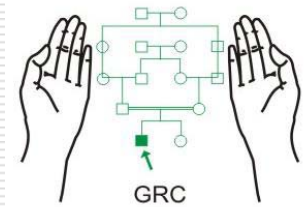
- Prenatal
 - Postnatal
 - Carrier screening
-

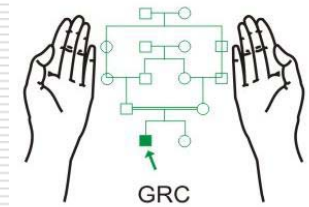
Genetic Diagnosis of Thalassaemia



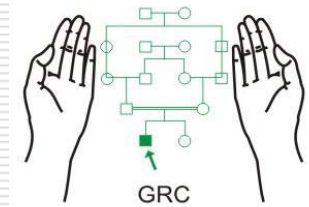
- Prenatal Diagnosis
 - Diagnosis in previously transfused patients
 - Silent thalassaemia alleles
 - Distinction between structural variants
 - Thalassaemia intermedia
 - α -thalassaemia
 - β -Thalassaemia carriers in certain situations
 - Rare thalassaemias
-

Prenatal Diagnosis

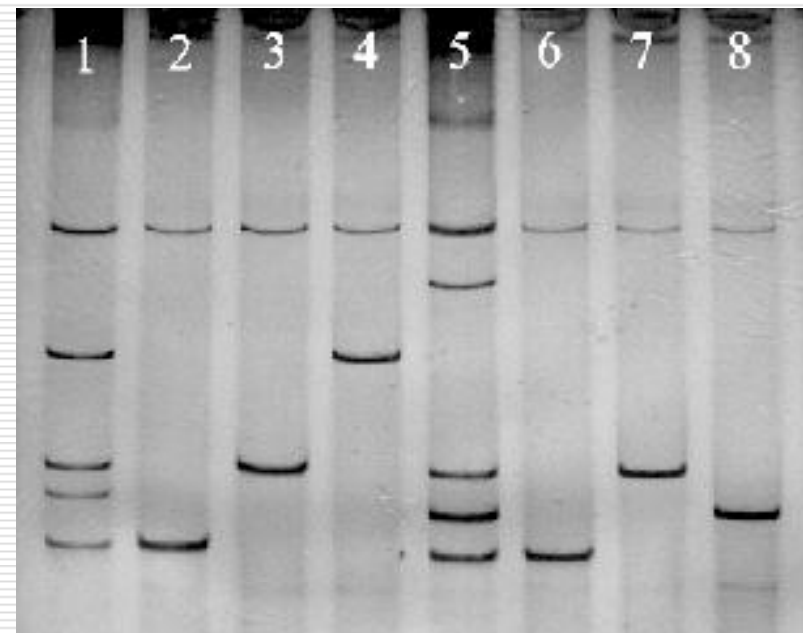




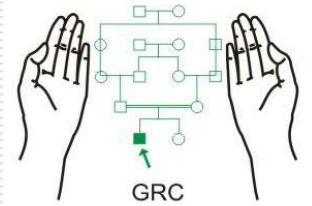
Mutation	Punjabi	Pathan	Sindhi	Baluchi	Mohajir	All
Common mutations						
IVSI-5 (G-C)	107 (27.2%)	27 (12.9%)	114 (43.9%)	131 (76.2%)	75 (41.4%)	454 (37.3%)
Fr 8-9 (+G)	146 (37.2%)	103 (49.1%)	29 (11.2%)	14 (8.1%)	23 (12.7%)	315 (25.9%)
Del 619 bp	14 (3.6%)	4 (1.9%)	36 (13.9%)	2 (1.2%)	29 (16.0%)	85 (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%)



<u>Primer ID:</u>	<u>Mutations Pooled:</u>	<u>Amplified Product size:</u>
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

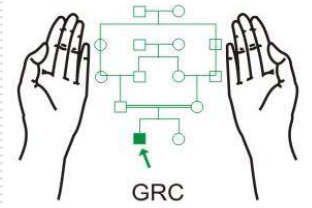


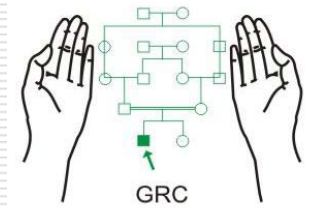
Fetal Sampling



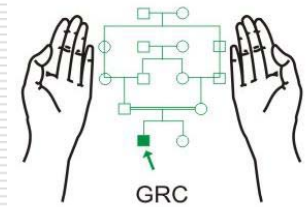
- Invasive techniques
 - Cord Blood Sampling
 - Amniocentesis
 - Chorionic Villus Sampling (CVS)
 - Non Invasive Techniques
 - Fetal cells in maternal blood
 - Fetal DNA in maternal blood
-

Chorionic Villus Sampling (CVS)

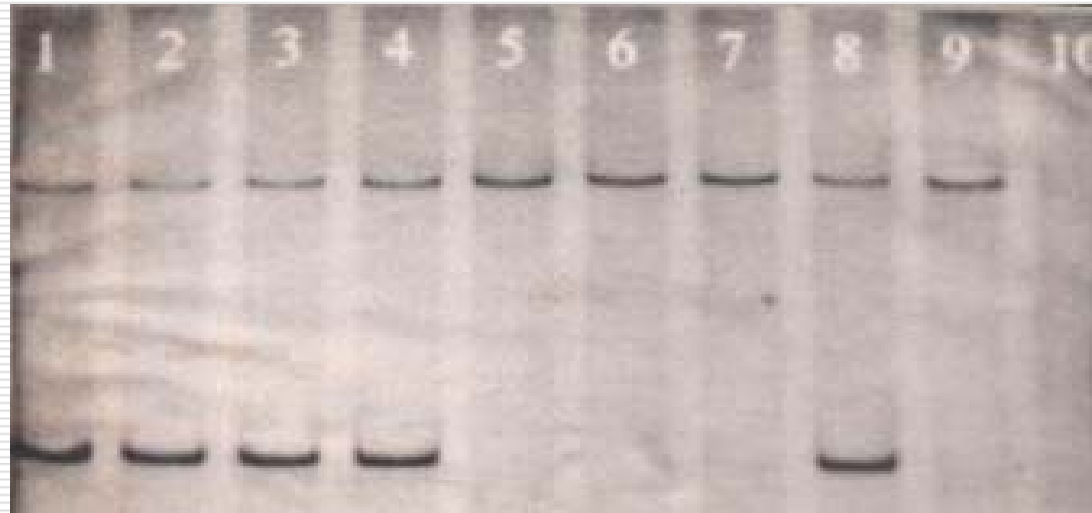




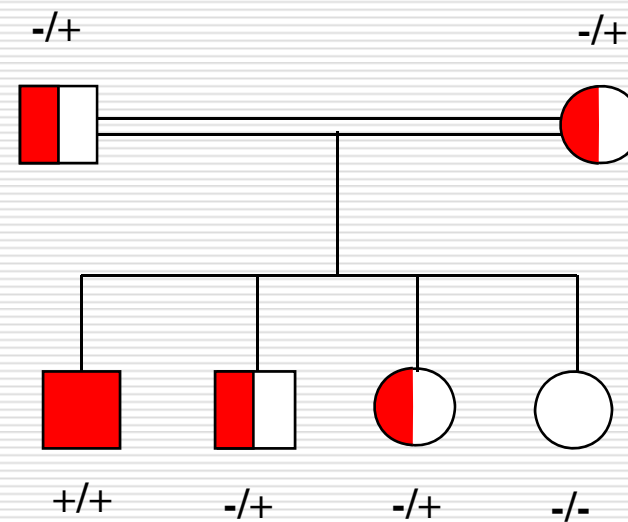
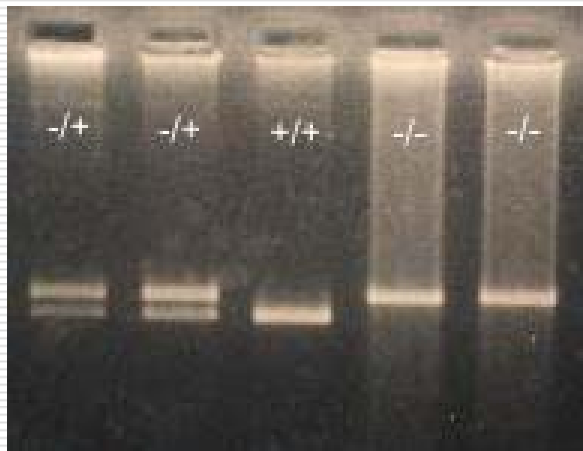
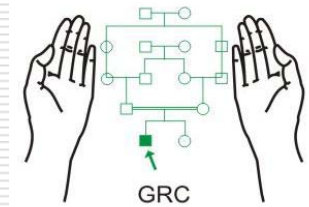
Prenatal Diagnosis by ARMS



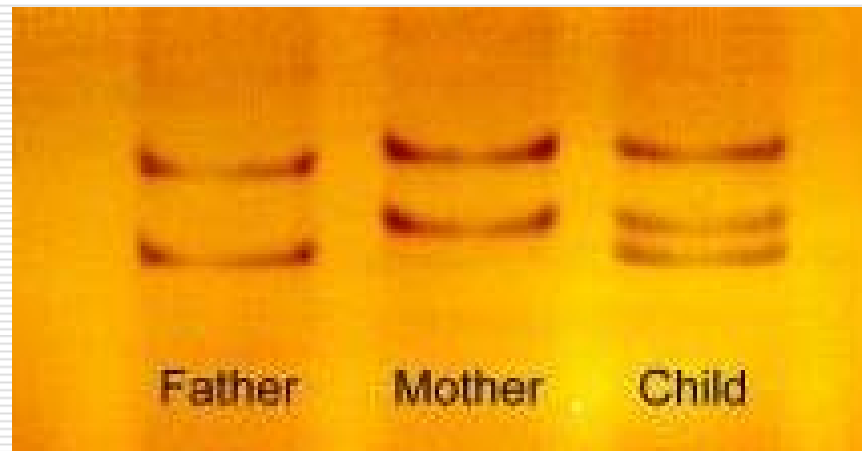
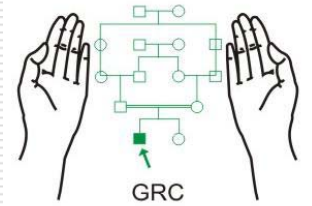
- Mutation
 1. Father
 2. Mother
 3. CVS
 4. CVS
 5. -ve
- Normal
 6. CVS
 7. CVS
 8. +ve
 9. -ve
 10. Blank



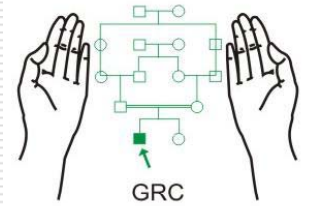
Prenatal Diagnosis by Linkage Analysis



Prenatal Diagnosis By Short Tandem Repeats (STR)

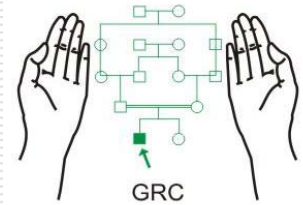


Misdiagnosis in PND ($<0.5\%$)



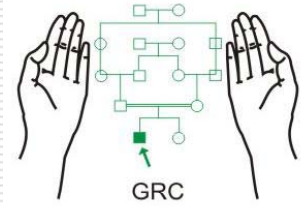
- Maternal Contamination in Fetal Sample
 - PCR Failure
 - Clerical Mistakes
 - Meiotic Crossover
-

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



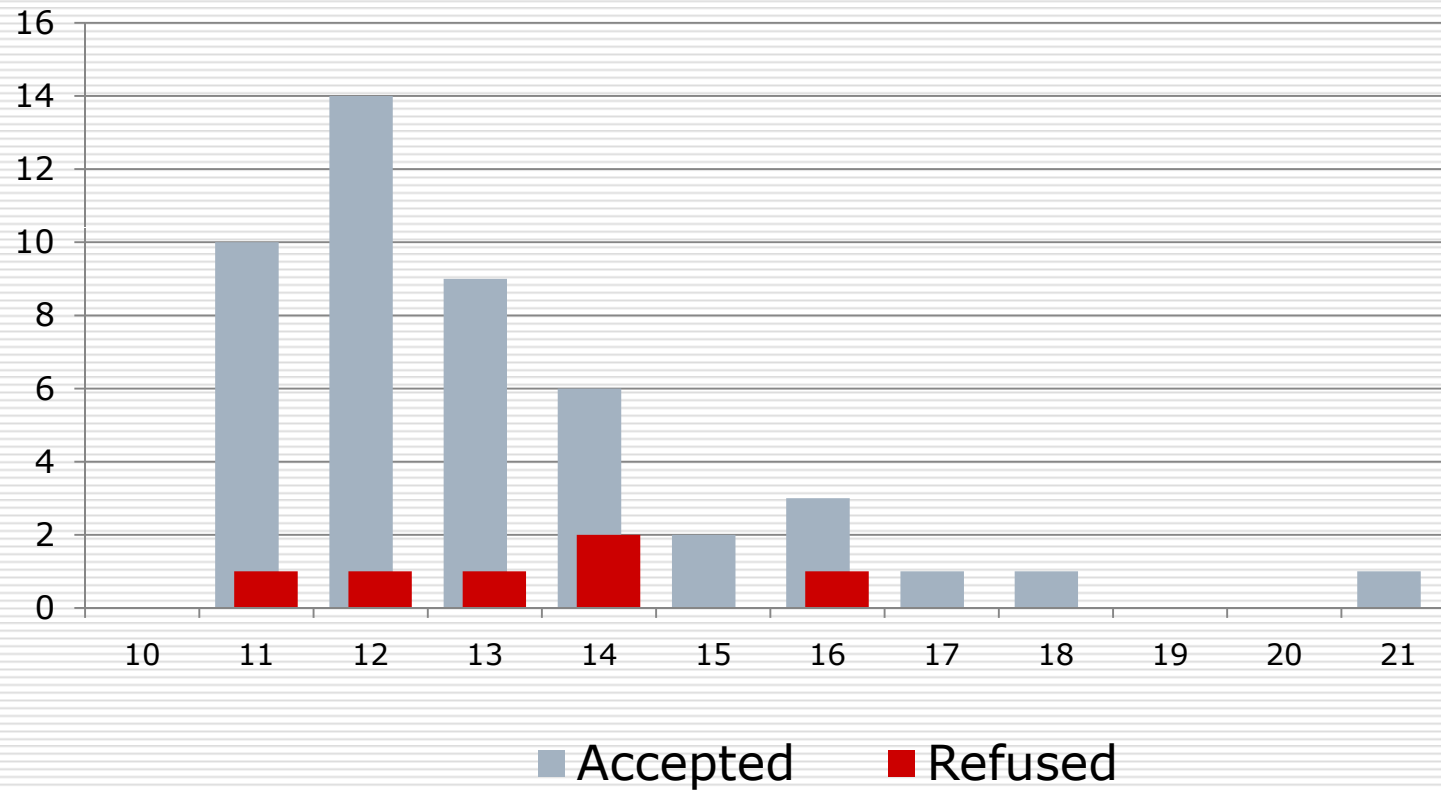
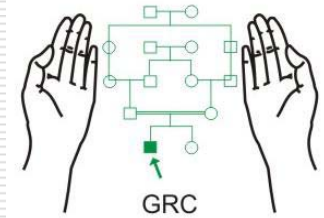
۱- حمل میں جب جان پڑ جائے (یعنی 14 دن بعد) تو اس وقت اس کی سب سے زیادہ طاقت
 ہے۔ اس لیے اس وقت تحقیق کرنا بہتر ہے کہ وہ حاملہ ہے یا نہیں۔ اگر حاملہ ہے تو اس کی
 جان پڑ جائے تو اس کی جان بچانے کے لیے اس وقت تحقیق کرنا بہتر ہے اور نوزائیدگی کو
 خواہ وہ کتنا ہی مستعد بیمار اور معذور کیوں نہ ہو تحقیق کرنا کسی صورت جائز
 نہیں ہے۔ اس لیے حمل میں جان پڑ جانے کے لیے اس وقت تحقیق کرنا اور کرنا بھی
 جائز نہیں ہے۔ بالخصوص جبکہ اس کا منہ بند ہے کہ طبی رپورٹ پر ہی حمل درست
 نہیں ہے یا رپورٹ درست ہو مگر پیدائش کے لیے اس وقت تک کہ اس سے یہ نتیجہ نکلے
 ہے۔

۲- البتہ حمل میں جان پڑنے سے قبل اگر طبی تحقیق سے حمل میں سبب
 بیماری یا نقص کا علم ہو جائے اور اسے انتہا پر غیب سے اسے اس وقت کا مسئلہ
 تو والدہ کیسے شرفیقا اسے ڈال گئی ہے۔

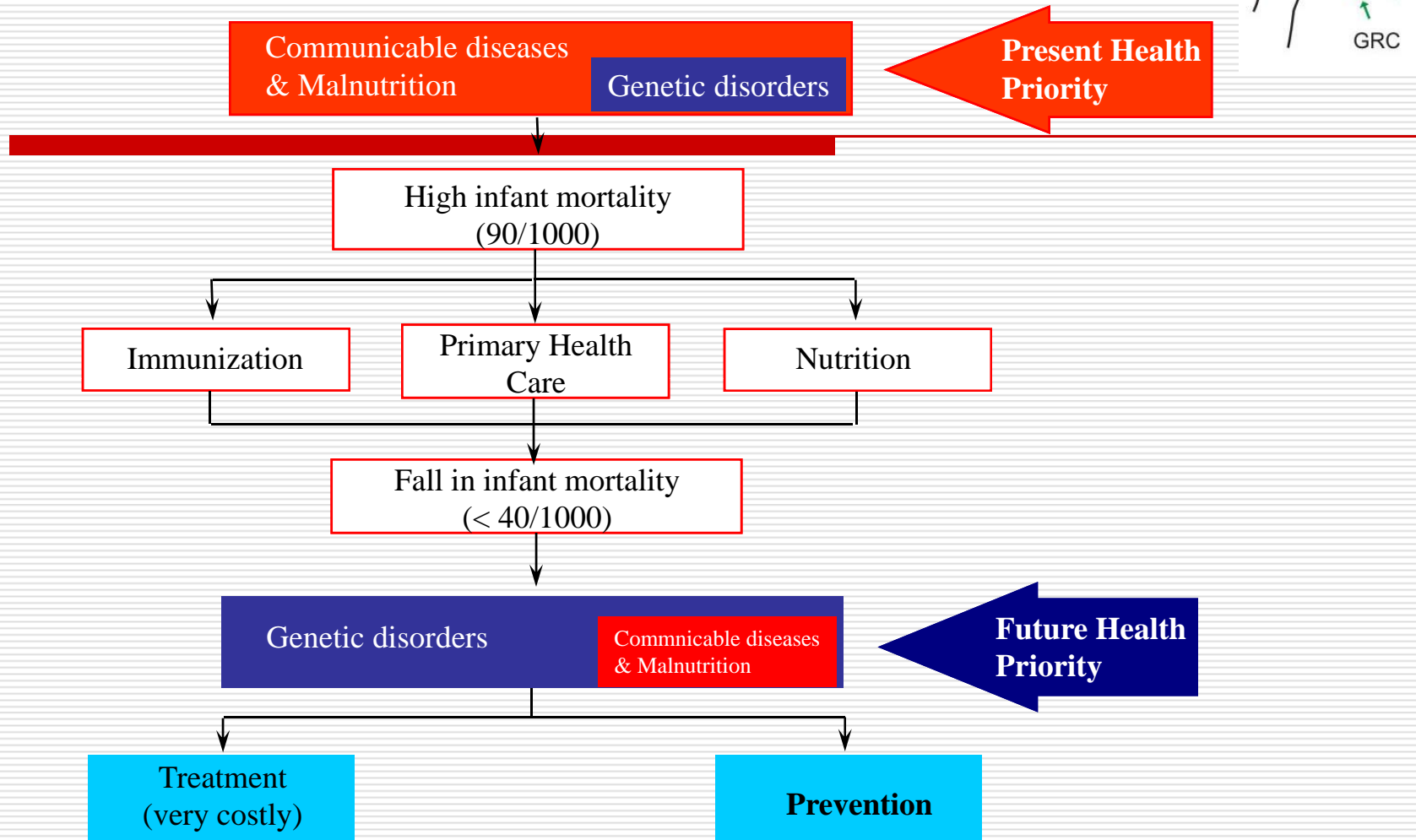
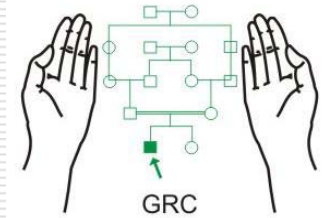
فان اولیٰ حیة تامل فی التضرع، حق بیاج ولاستطاع لہد الحلی نعم بیاج حالہ
 یصلح منہ شیء ولن یکن زلات الا بعد ما تہد بعشرین یوما وھذا یقتضی انھم
 انوارہا بالتعلیق نفع الروح وراؤ نہو فظا لظن التعلیق یتحقق بالثبوت قبل عذرہ
 المیتۃ کذا من الفسخ والھذا تم ینبہ عدم توقف جواز استعمالھا قبل المیتۃ المذکورہ
 علی اذن الزوج۔ وھذا کہ الھجۃ الی نیتہ ولا أقول بالولی ذالما امرن لکسر بیض العید
 فعمدہ لأنہ اصل العید فعمدہ کان یؤخذ بالجزء فلا یؤخذ من ان البقیۃ ثم عدا
 اذا استقلت البیض عنہ قال ابن وہبان ومن الأقدار ان یتخلع لہنہا لولہا
 الحلی ولیس تکف العصبی ما لہا جرمہ العشر ویناف عدلہ کہ سئل عنہ

شہدہ محمد عرفان علی منہ
 دارالافتاء - جامعہ دارالعلوم کراچی
 ۱۳۴۵/۹/۱۸
 ۱۹ - ۹ - ۲۰۲۵
 محمد عرفان علی
 دارالافتاء
 دارالعلوم کراچی

Termination of Pregnancy

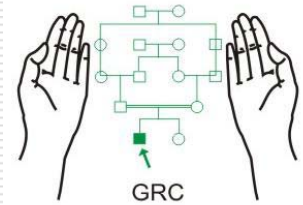


(S. Ahmed et al 2000)



(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
-