Prenatal Diagnosis of Thalassaemia

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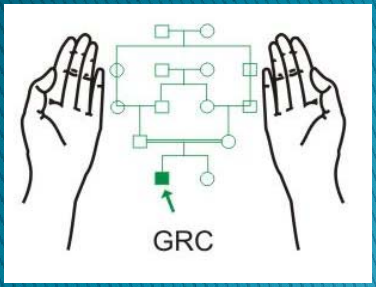
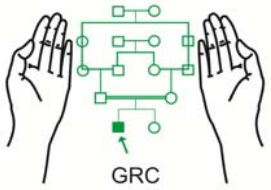
MBBS; MCPS; FCPS (Pak); PhD (London)

Genetics Resource Centre (GRC)

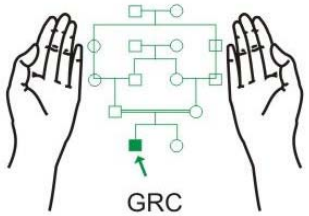


www.grcpk.com

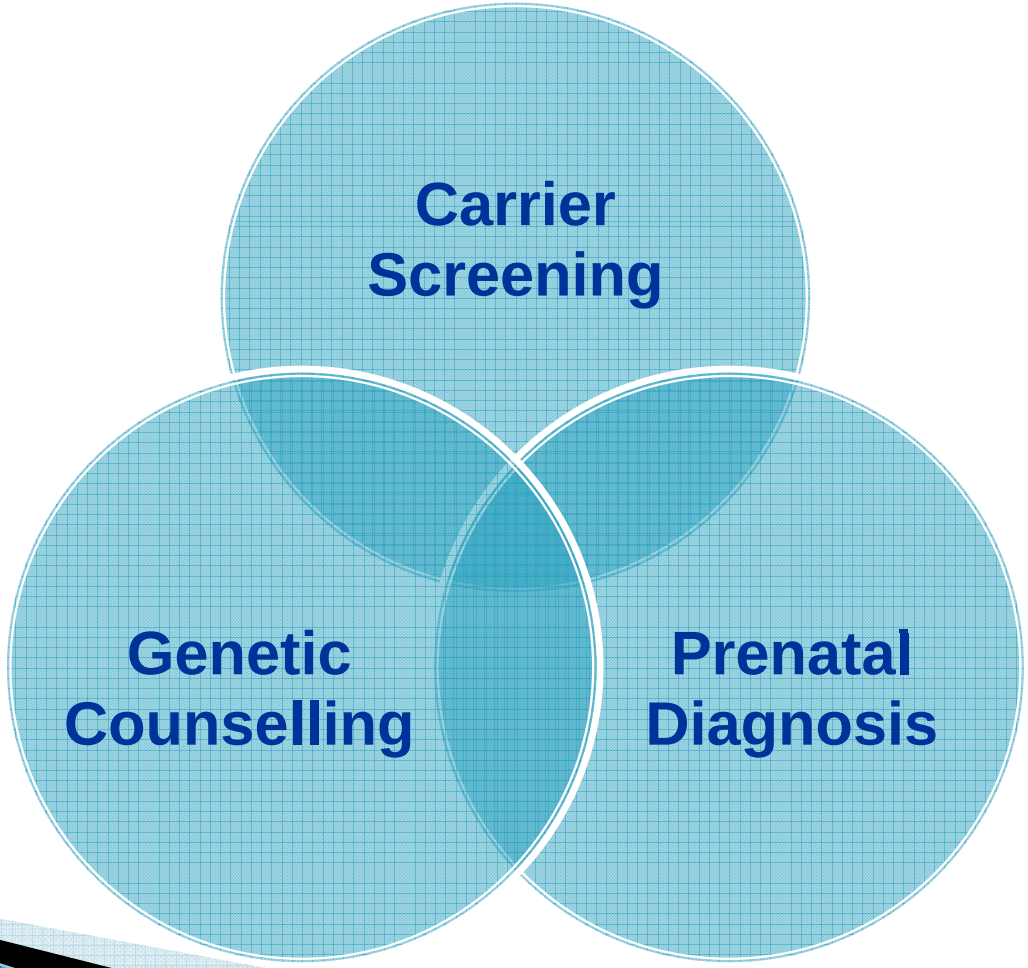
Prenatal Diagnosis (PND)

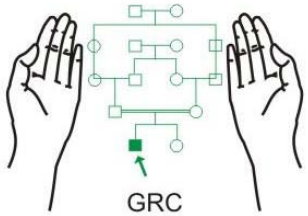


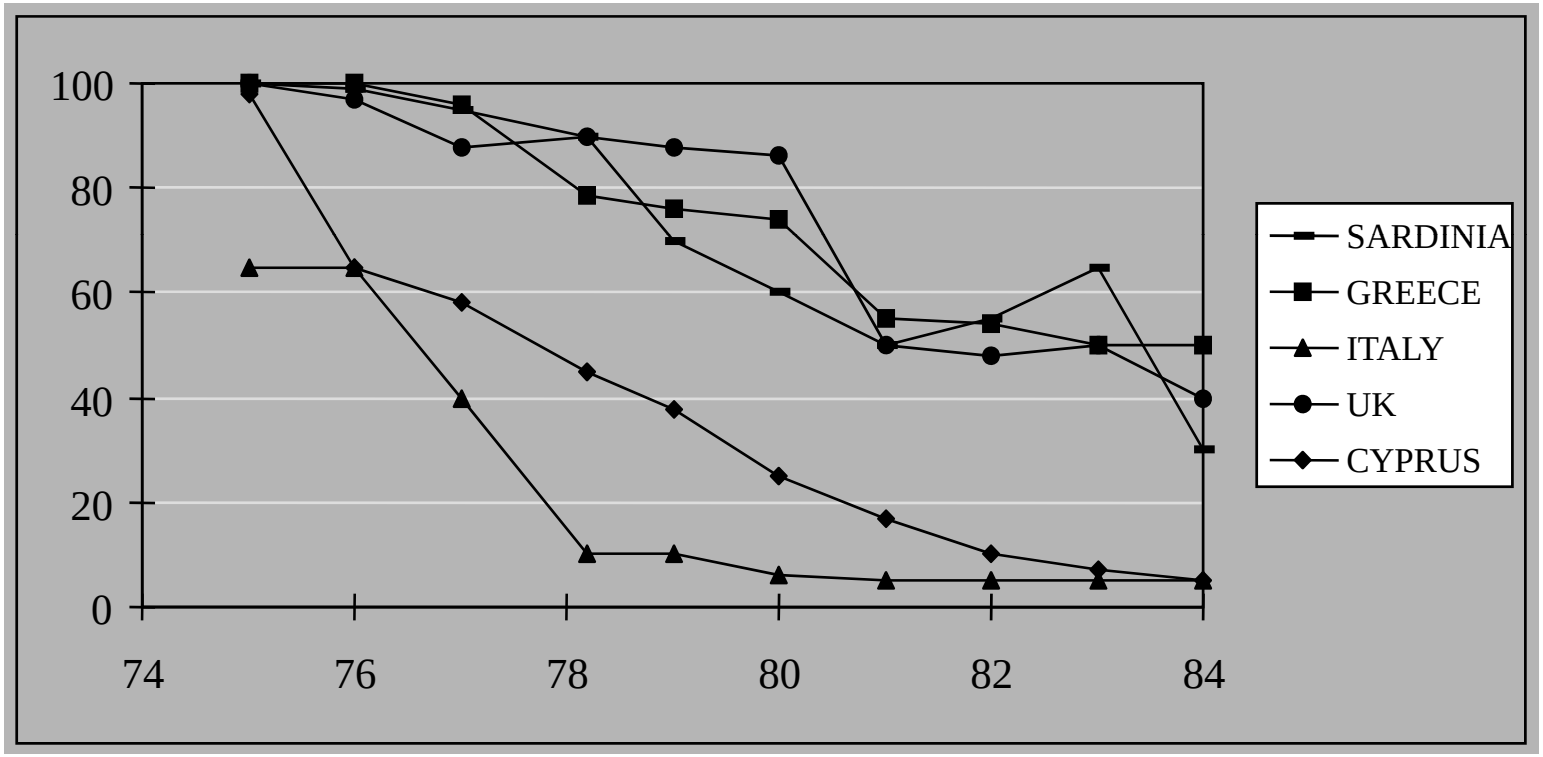
- Testing for Thalassaemia in a fetus or embryo before it is born
- Informed choice for the parents
- Outcomes
  - Unaffected fetus
  - Affected fetus
    - Accepted
    - Termination of Pregnancy



Prevention of Genetic Disorders



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80
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40
20
0
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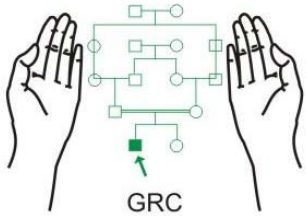
80
82
84
SARDINIA
GREECE
ITALY
UK
CYPRUS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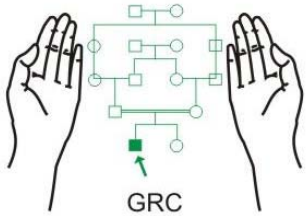
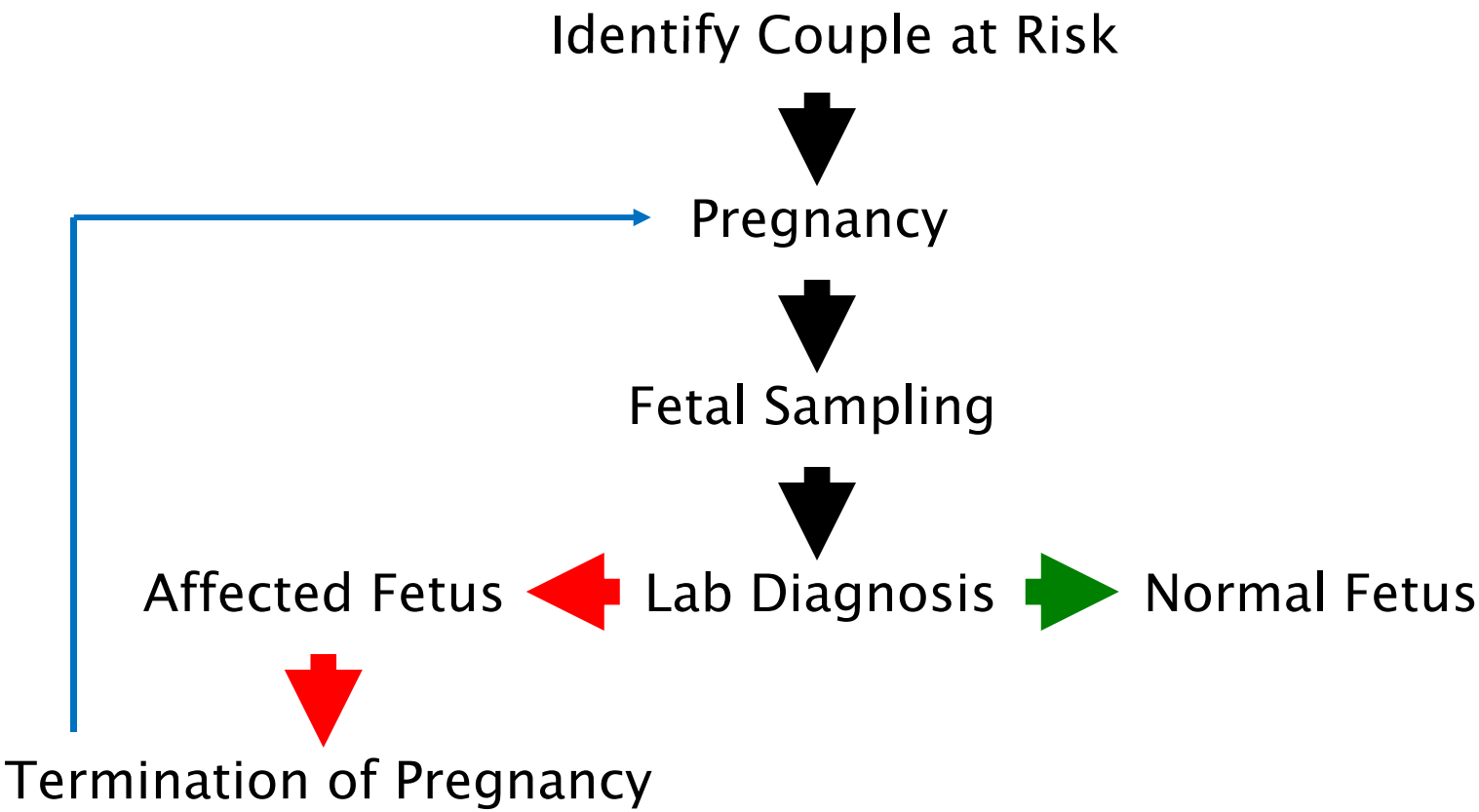
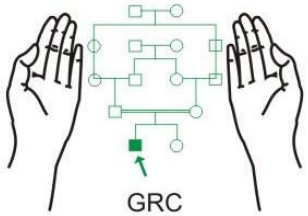
Historical Background

- Fetal blood sampling (early eighties)
- Chorionic villus sampling (Giuseppe Simoni, 1983)
- Maternal blood



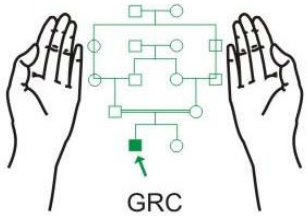
Prenatal Diagnosis





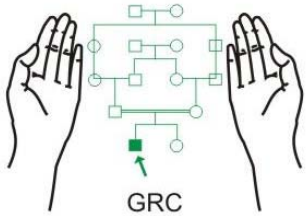
Prenatal Diagnosis

- The Couple at Risk
- Fetal Sampling
- Lab Testing

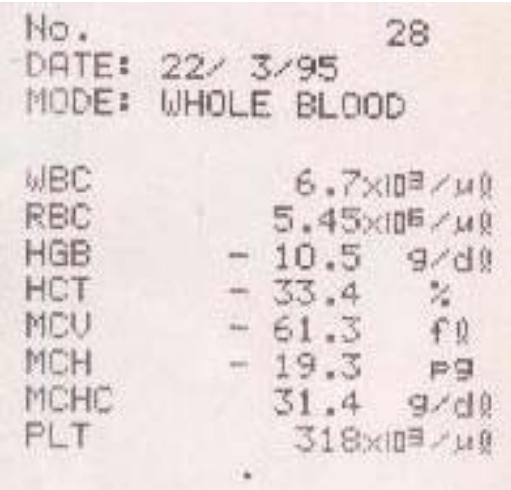


Couples at Risk

- Retrospective identification)
  - Couples with previously affected children
- Prospective identification
  - Positive screening tests

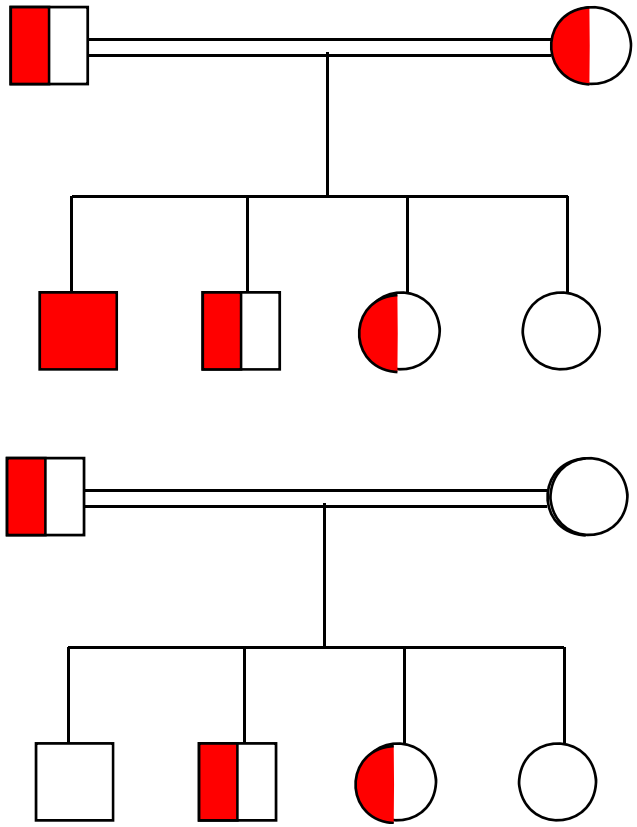


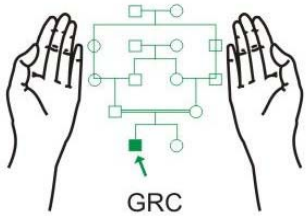
Who needs Prenatal Diagnosis? (Couples at Risk)



No. 28
DATE: 22/ 3/95
MODE: WHOLE BLOOD

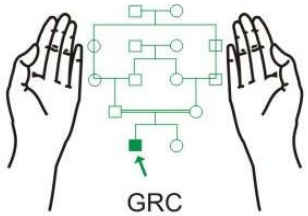
WBC	6.7	$\times 10^3/\mu l$
RBC	5.45	$\times 10^6/\mu l$
HGB	- 10.5	g/dl
HCT	- 33.4	%
MCV	- 61.3	fl
MCH	- 19.3	pg
MCHC	31.4	g/dl
PLT	318	$\times 10^3/\mu l$





Fetal Sampling

- Cord blood sampling
- Amniocentesis
- Chorionic Villus Sampling (CVS)
- Maternal Blood



Chorionic Villus Sampling (CVS)

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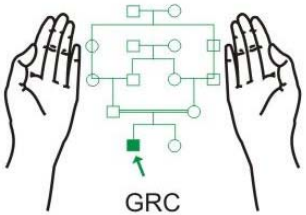
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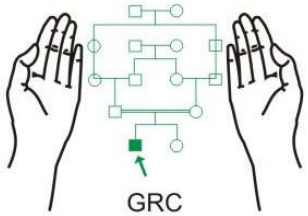
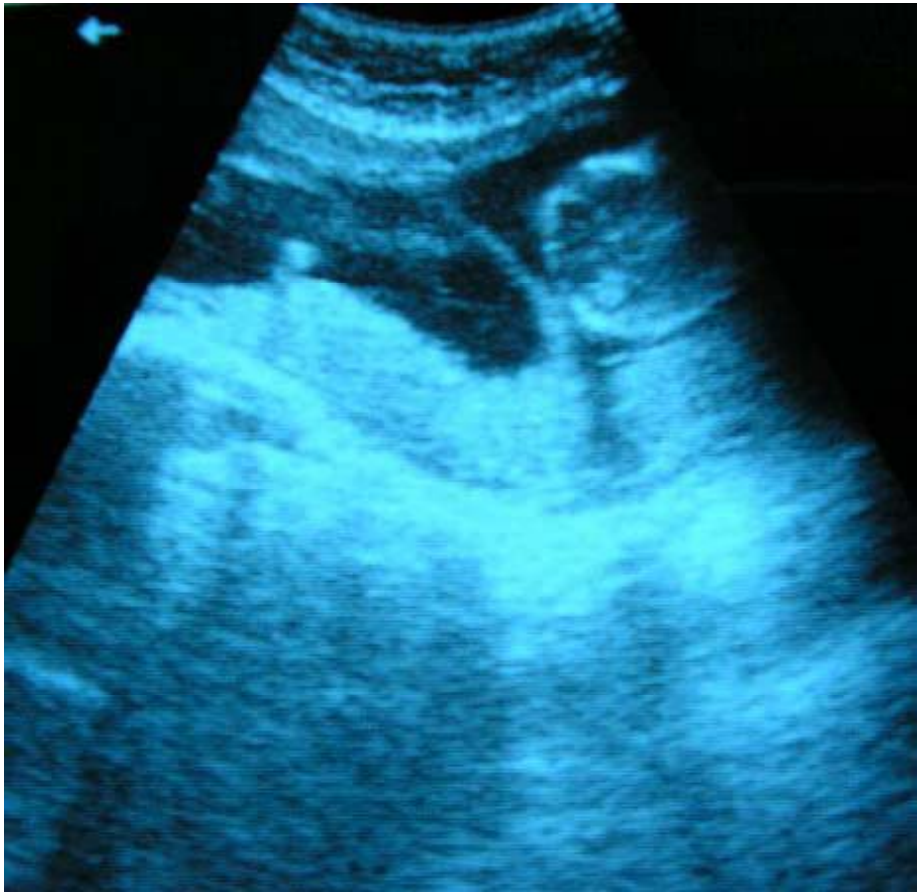
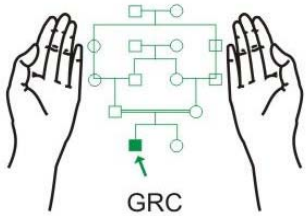
Sex:♂ Age: Dr:


GRC

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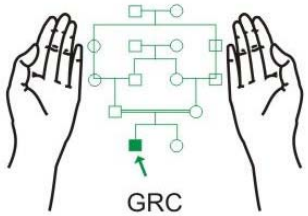
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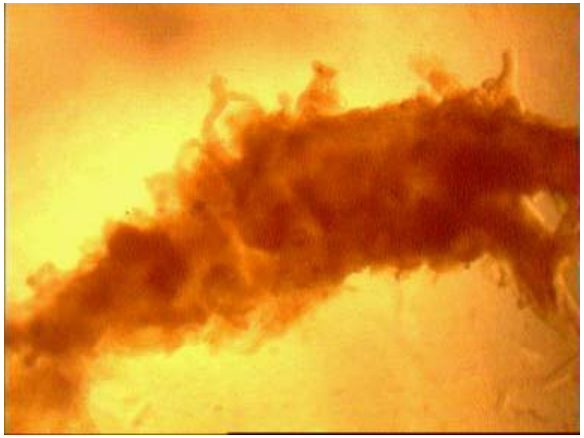

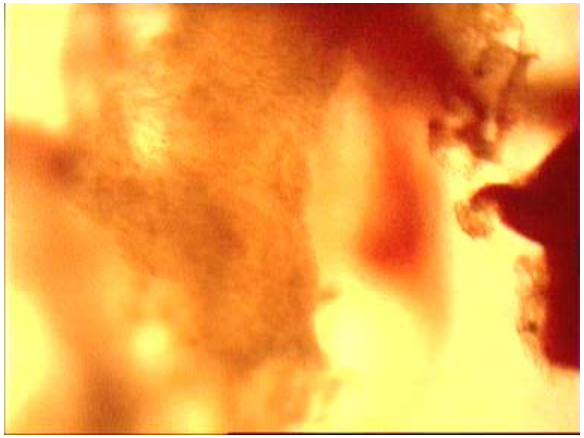

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Lab Diagnosis

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%)

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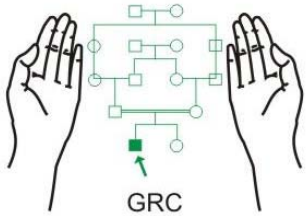
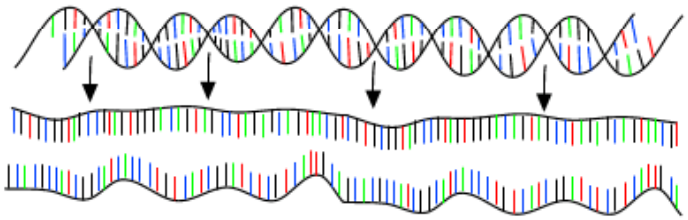


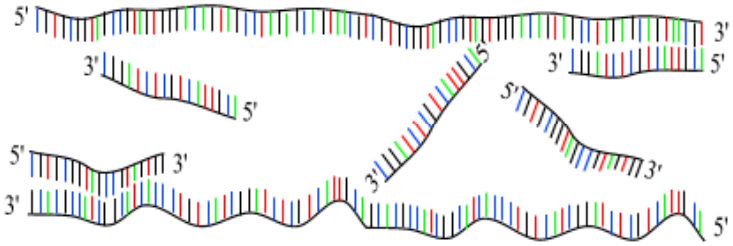
PCR : Polymerase Chain Reaction

30 - 40 cycles of 3 steps :



Step 1 : denaturation

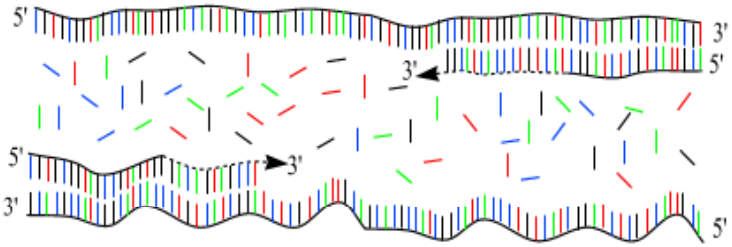
1 minut 94 °C



Step 2 : annealing

45 seconds 54 °C

forward and reverse primers !!!

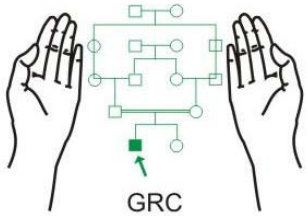


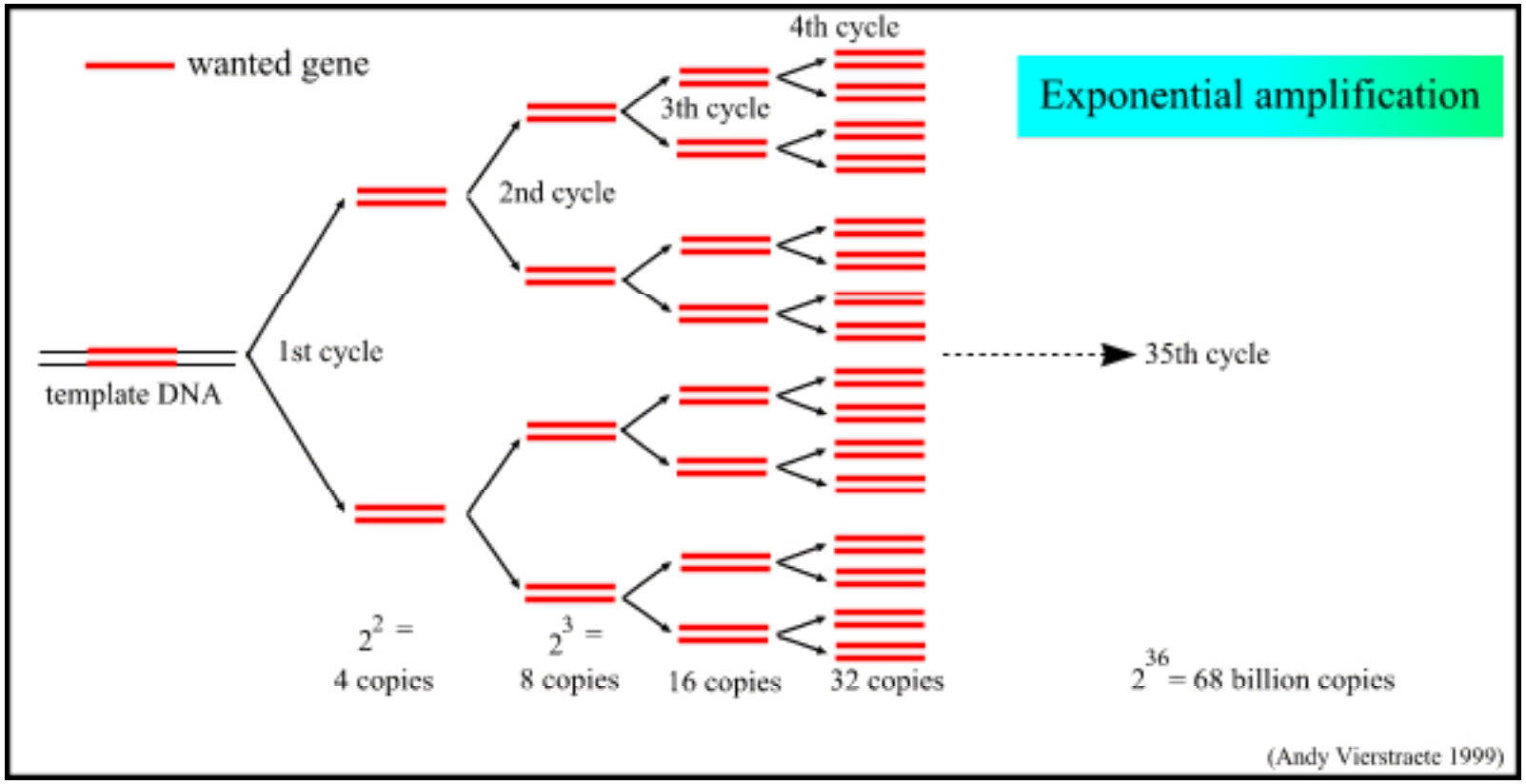
Step 3 : extension

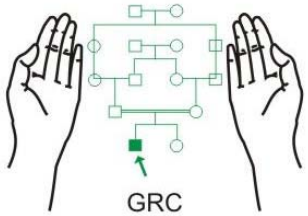
2 minutes 72 °C

only dNTP's

(Andy Vierstraete 1999)




wanted gene
Exponential amplification
4th cycle
3th cycle
2nd cycle
1st cycle
template DNA
35th cycle
2^2 =
4 copies
2^3 =
8 copies
16 copies
32 copies
2^{36} = 68 billion copies
(Andy Vierstraete 1999)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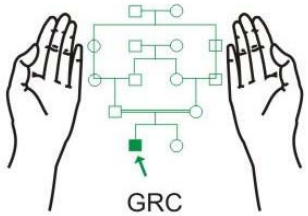
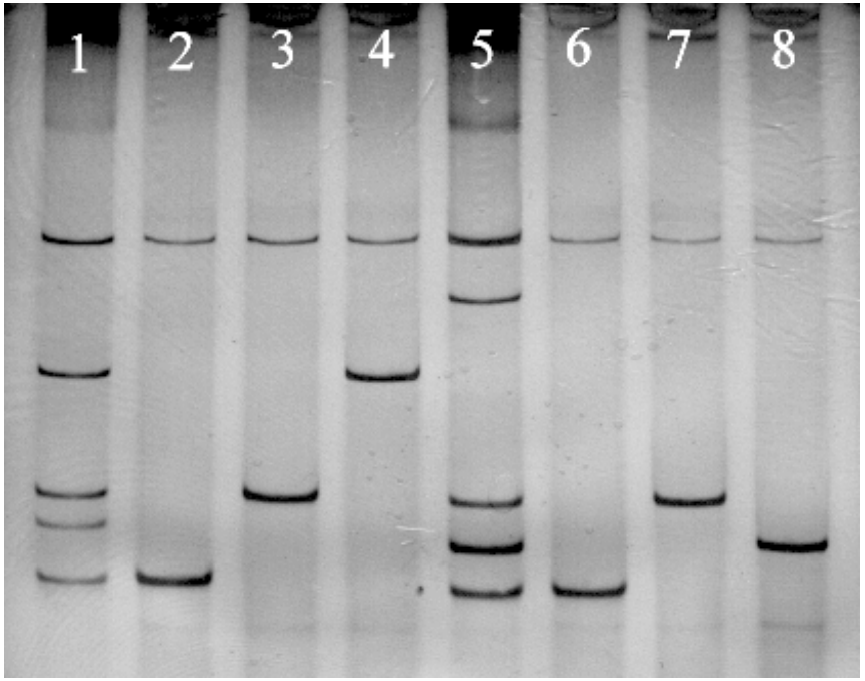
Molecular Methods in PND

- Mutation Analysis
  - Specific methods
    - ARMS
    - Dot Blot & Reverse Dot Blot
    - Sequencing
  - Non specific methods
    - DGGE
    - SSCP
- Linkage analysis



Multiplex ARMS PCR

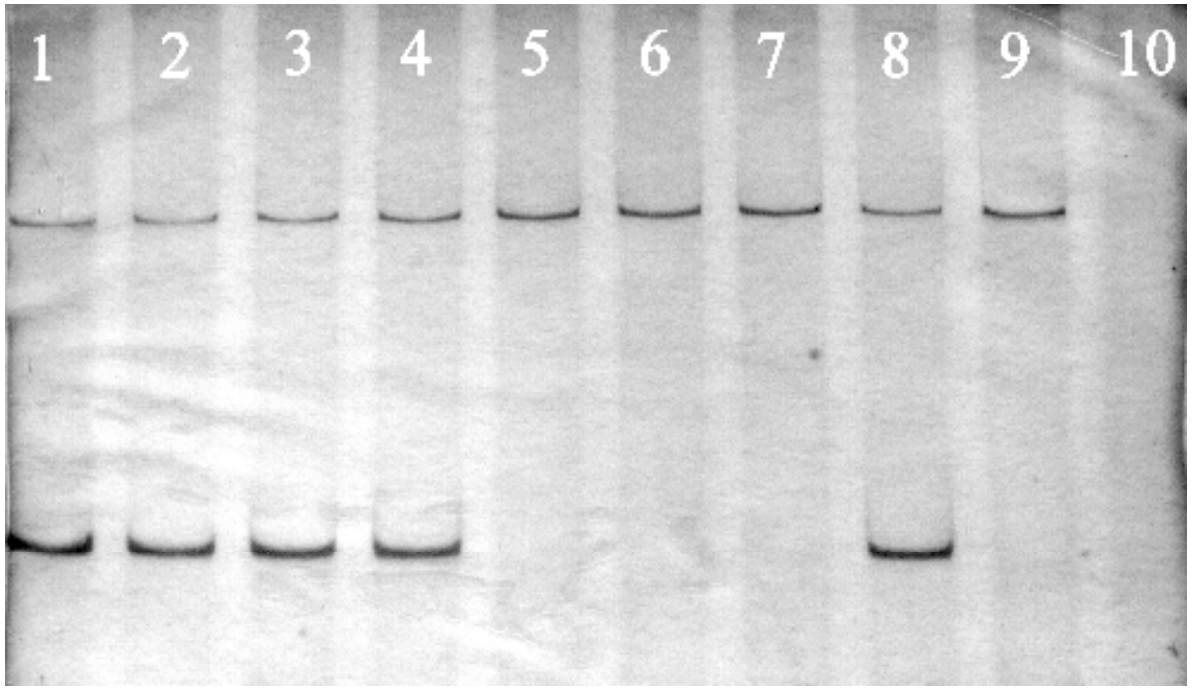


(Ahmed et al, Prenatal Diagnosis 2000)

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

Prenatal Diagnosis by ARMS

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



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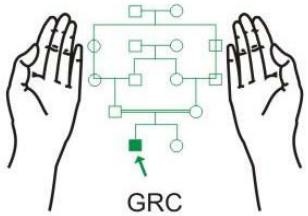
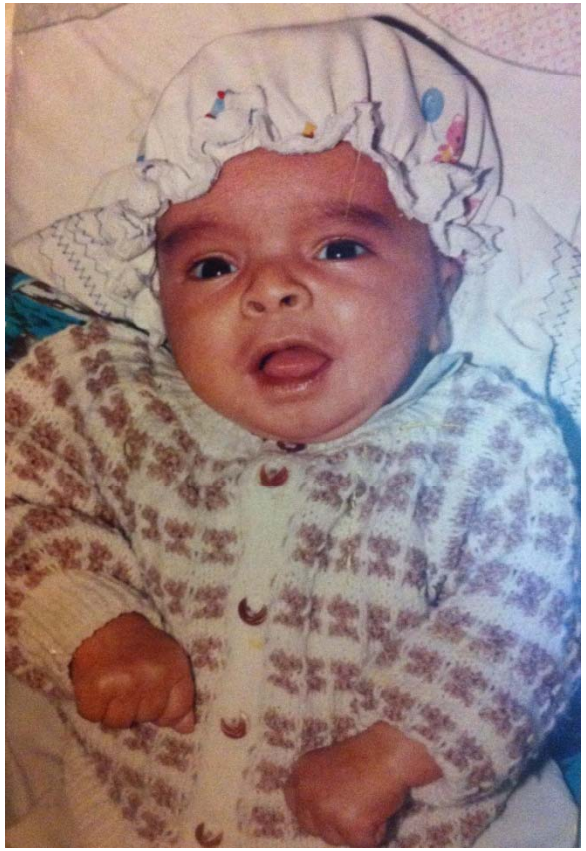
Prenatal Diagnosis in Pakistan

- First Prenatal Diagnosis
  - 1st may 1994 at AFIP
- Over 1000 each year
  - AFIP Rawalpindi
  - AKU Karachi
  - PNS Shifa Karachi
  - NIBD Karachi
  - NIBGE Faisalabad
  - GRC Rawalpindi



PND in Pakistan (1st may 1994)



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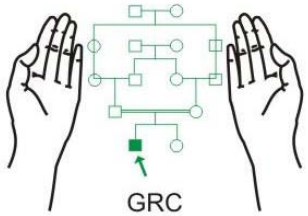
Cost of Prenatal Diagnosis

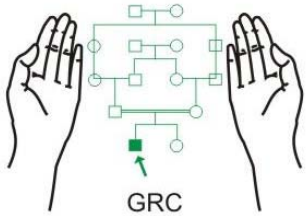
- UK £500 (Rs. 80,000)
- Pakistan (GRC): Rs. 5500
  - Indigenous PCR kits
  - Multiplexing in PCR
  - Indigenous PCR equipment



Prenatal Diagnosis: Reasons for under-utilization

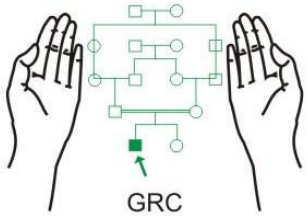
• Did not know	23%
• Expensive	23%
• No access	17%
• Delay in seeking	16%
• Advised against	12%
• Religious beliefs	9%
• Other reasons	9%

(S. Naseem et al, 2008)



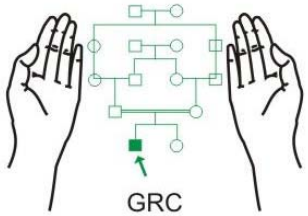
Prenatal Diagnosis in Pakistan

- Required: 20,000/year
- Done: 1000/year (5.0%)



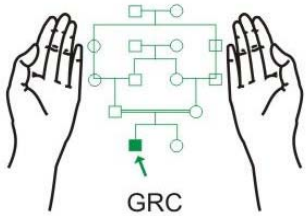
Prenatal Diagnosis

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



Misdiagnosis in Prenatal Diagnosis (<0.5%)

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



Termination of Pregnancy

۱- حمل میں جب جان پڑ جائے (یعنی ۱۲۰ دن بعد) تو اسقاط حمل کی شرعاً اجازت نہیں دی گئی ہے، اگرچہ طبی تحقیق کے ذریعہ حمل میں کسی بیماری کا خطرہ غالب ہی کیوں نہ ہو جائے، کیونکہ جان پڑنے کے بعد اسقاط حمل قتل کے مشابہ ہے اور زندہ بچہ کو خواہ وہ کتنا ہی شدید بیمار اور معذور کیوں نہ ہو قتل کرنا کسی صورت جائز نہیں ہے۔ اسی طرح حمل میں جان پڑ جانے کے بعد اسقاط کرنا اور کروانا بھی جائز نہ ہوگا، بالخصوص جبکہ امکان موجود ہے کہ طبی رپورٹ پوری طرح درست نہ ہو یا رپورٹ درست ہو مگر پیدائش کے بعد اللہ کے حکم سے بچہ تندرست ہو۔

۲- البتہ حمل میں جان پڑنے سے قبل اگر طبی تحقیق سے حمل میں شدید بیماری یا نقص کا علم ہو جائے اور ماہر ڈاکٹر طبیب کے اسقاط کا مشورہ دے تو والدہ کیلئے شرعاً اسقاط کی گنجائش ہے۔

فی الشامیۃ: قال فی النہر لقائل ان یباح الاسقاط بعد الحمل نعم یباح ما لم یتصل منہ شیئ وان یکون ذلک الی تمام مائۃ وعشرین یوما وھذا یقتضی انہم اطلقوا التخلیق نفخ الروح والا فھو غلط لان التخلیق یتحقق بالمائۃ الاولی قبل ھذہ المدۃ کذا فی الفتح والظاھر انہ یفید عدم توقف جواز اسقاطھا قبل المدۃ المذکورۃ علی اذن الزوج۔ وفی کراھۃ الخانیۃ ولا أقول بالحل اذا المرأۃ لو کسر بیض الصید ضمنہ لانہ اصل الصید فلما کان لا یؤاخذ بالجزاء فلا أقل من أن یلحقھا إثم ھنا اذا اسقطت لغیر عذر۔ قال ابن وھبان ومن الأعذار أن ینقطع لبنھا لعدم ظہور الحمل ولیس لأب الصبی مال یرضع بہ الغیر ویخاف ھلاکہ۔ شامی ج۱ ص۱۷۶

بندہ محمود اشرف عفی عنہ
دار الافتاء - جامعہ دار العلوم کراچی
۱۴۱۵/۹/۱۸

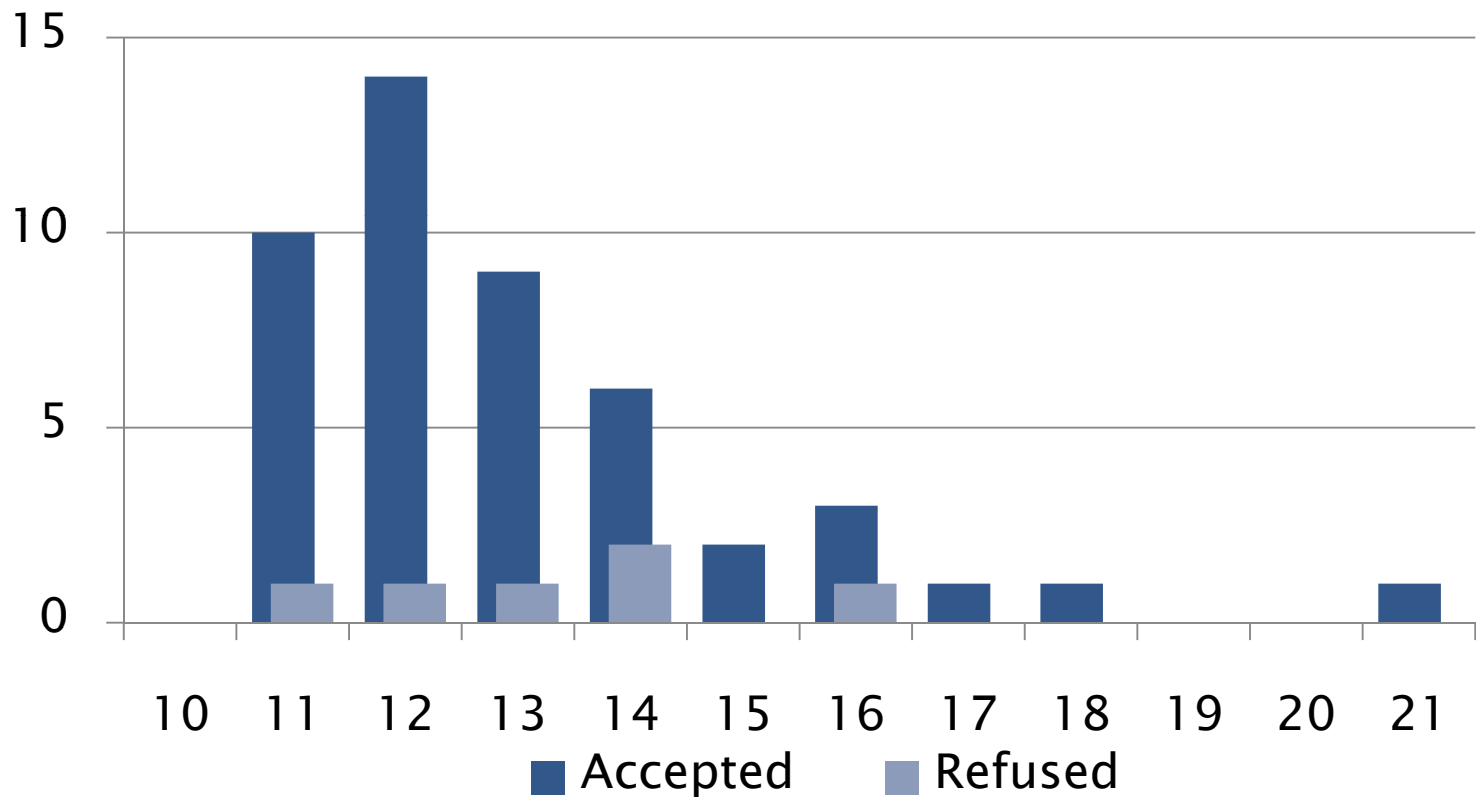
الجواب صحیح
۱۹-۹-۱۴۱۵

دار الافتاء جامعہ دار العلوم کراچی

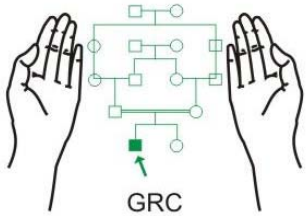
مفتی

GRC

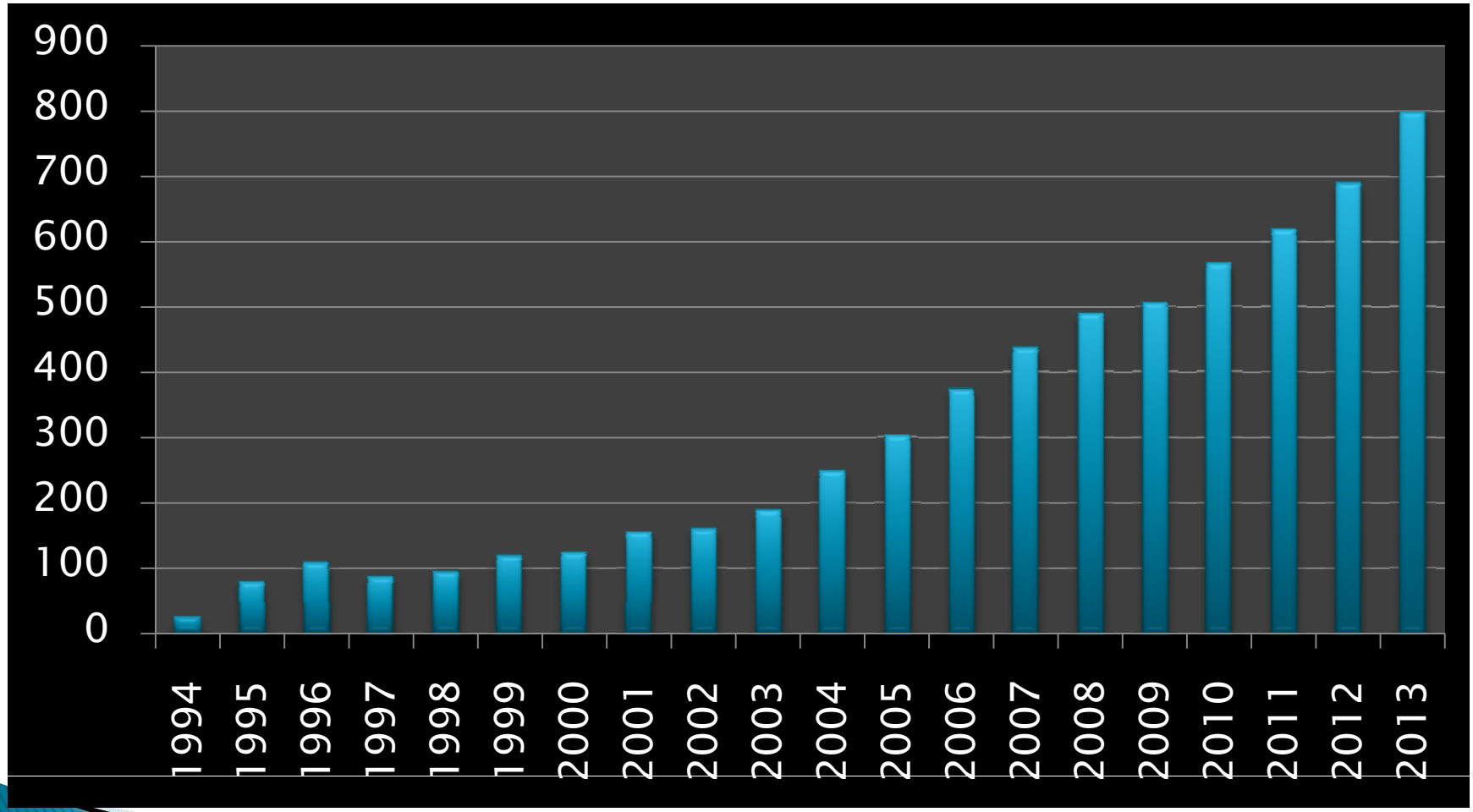
Termination of Pregnancy for Thalassaemia



Ahmed et al, 2000



PND for Thalassaemia in Pakistan
Armed Forces Institute of Pathology (AFIP)
Genetic Resource Centre (GRC)



GRC