PCR in Inherited Disorders

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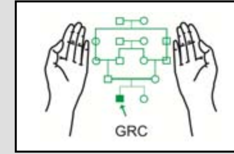


The PCR technology has enabled even small labs to develop expertise in the diagnosis of common inherited disorders. The most useful application of PCR in such situations is the prenatal diagnosis (PND). There is a long list of inherited disorders where PCR has a significant role to play. A typical example is β-thalassaemia. It is common and its molecular genetics is also well known. Trisomies, although not inherited in most situations, are other major areas where PCR can play useful role in rapid prenatal diagnosis.

In this chapter molecular diagnosis of β-thalassaemia is described as an example. The diagnosis of other single gene disorders caused by point mutations e.g. cystic fibrosis etc. can also be done on similar lines.

β-thalassaemia

There are over 200 different mutations that cause β-thalassaemia. However, each ethnic population has its own set of common and uncommon mutations. The first step in carrying out PCR based diagnosis of β-thalassaemia is to know the pattern of mutations in the target population. This knowledge is essential for genetic diagnosis.

Screening for β-thalassaemia mutations:

Most of the β-thalassaemia mutations are point mutations that are best detected by ARMS (Chapter 7). ARMS can be done in separate reactions for each mutation or as multiplex PCR.

Screening strategy

Standard ARMS (separate reactions)

1. In the first round of PCR common mutations are tested.
2. The samples that do not show any of the common mutations are then tested in the next rounds for the uncommon and the rare mutations.
3. The list and the sequences of primers for the β-thalassaemia mutations in the major world populations are given in Table 13.1. For convenience the primers may be given a serial number. The numbers are easier to remember and document than the full nomenclature of the allele.
4. In each reaction the ARMS primer for a mutation is run with another primer called "common primer".
5. Most of the ARMS primers are designed as complementary to the forward strand of DNA. All of these primers are used with the same "common primer" which is complementary to the reverse strand (primer 3).

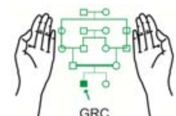
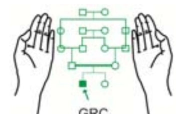


Table 13.1. ARMS primers for β-thalassaemia mutations in the major world populations. The sequence of primers for the mutant and the normal alleles are given. Each ARMS primer is used with one of the common primers. (No. 3 & 4). The amplified product size is also given. Primers 1 and 2 are used to amplify 861bp PCR internal control fragment of the distal portion of β-globin gene.

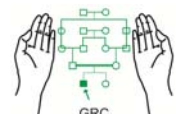
Control and common primers:			
1. Control-F	5'-CAATGTATCATGCCTCTTTGCACC		
2. Control-R	5'-GAGTCAAGGCTGAGAGATGCAGGA		
3. Common-1	5'-ACCTCACCCTGTGGAGCCA		
4. Common-2	5'-CCCCTTCCTATGACATGAACTTAA		
Allele	**Primer sequence**	**Used with**	**Product Size**
Asian mutations			
Fr 8-9 (+G) M	5'-CCTTGCCCCACAGGGCAGTAACGGCACACC	3	215
Fr 8-9 N	5'-CCTTGCCCCACAGGGCAGTAACGGCACACT	3	215
IVSI-5 (G-C) M	5'-CTCCTTAAACCTGTCTTGTAACCTTGTTAG	3	285
IVSI-5 N	5'-CTCCTTAAACCTGTCTTGTAACCTTGTTAC	3	285
Fr 41-42 (-TTCT) M	5'-GAGTGGACAGATCCCCAAAGGACTCAACCT	3	439
Fr 41-42 N	5'-GAGTGGACAGATCCCCAAAGGACTCAAAGA	3	443
Del 619bp-F	5'-CAATGTATCATGCCTCTTTGCACC	2	242
Del 619bp-R	5'-GAGTCAAGGCTGAGAGATGCAGGA	1	242
Cd 15 (G-A) M	5'-TGAGGAGAAGTCTGCCGTTACTGCCCAGTA	4	500
Cd 15 N	5'-TGAGGAGAAGTCTGCCGTTACTGCCCAGTG	4	500
Cd 5 (-CT) M	5'-ACAGGGCAGTAACGGCAGACTTCTCCGCGA	3	205
Cd 5 N	5'-ACAGGGCAGTAACGGCAGACTTCTCCGCAG	3	205
IVSI-1 (G-T) M	5'-TTAAACCTGTCTTGTAACCTTGATACGAAA	3	281
IVSI-1 N	5'-GATGAAGTTGGTGGTGAGGCCCTGGGTAGG	4	450
Cd 30 (G-C) M	5'-TAAACCTGTCTTGTAACCTTGATACCTACG	3	280
Cd 30 (G-A) M	5'-TAAACCTGTCTTGTAACCTTGATACCTACT	3	280
Cd30 N	5'-TAAACCTGTCTTGTAACCTTGATACCTACC	3	280
Fr 16 (-C) M	5'-TCACCACCAACTTCATCCACGTTCACGTTC	3	238
Fr 16 N	5'-TCACCACCAACTTCATCCACGTTCACGTTG	3	239
IVSII-1 (G-A) M	5'-AAGAAAACATCAAGGGTCCCATAGACTGAT	3	634
IVSII-1 N	5'-AAGAAAACATCAAGGGTCCCATAGACTGAC	3	634
Cap+1 (A-C) M	5'-ATAAGTCAGGGCAGAGCCATCTATTGGTTC	4	567
Cd 48 (+ATCT) M	5'-ATAACAGCATCAGGAGTGGACAGATAGATC	3	467
IVSI-25 M	5'-CTCTGGGTCCAAGGGTAGACCACCAGCATA	3	354
-88 (C-T) M	5'-TCACCTAGACCTCACCCTGTGGAGCCTCAT	4	655
Mediterranean mutations			
IVSI-110 M (G-A)	5'-ACCAGCAGCCTAAGGGTGGGAAAATAGAGT	3	390
IVSI-110 N	5'-ACCAGCAGCCTAAGGGTGGGAAAATACACC	3	390
IVSI-1 (G-A) M	5'-TTAAACCTGTCTTGTAACCTTGATACGAAT	3	281
IVSI-1 N	5'-TTAAACCTGTCTTGTAACCTTGATACGAAC	3	281
C 39 M (C-T)	5'-CAGATCCCCAAAGGACTCAAAGAACCTGTA	3	436
C 39 N	5'-TTAGGCTGCTGGTGGTCTACCCTTGGTCCC	4	436
IVSI-6 M (T-C)	5'-TCTCCTTAAACCTGTCTTGTAACCTTCATG	3	286
IVSI-6 N	5'-TCTCCTTAAACCTGTCTTGTAACCTTCATA	3	286
IVSII-1M (G-C)	5'-AAGAAAACATCAAGGGTCCCATAGACGCAG	3	634
IVSII-1 N	5'-AAGAAAACATCAAGGGTCCCATAGACTGAC	3	634
IVSII-745 M (C-G)	5'-TCATATTGCTAATAGCAGCTACAATCGAGG	2	738
IVSII-745 N	5'-TCATATTGCTAATAGCAGCTACAATCGAGC	2	738
Far Eastern mutations			
Fr 41-42 (-TTCT) M	5'-GAGTGGACAGATCCCCAAAGGACTCAACCT	3	439
Fr 41-42 N	5'-GAGTGGACAGATCCCCAAAGGACTCAAAGA	3	439
C17 M (A-T)	5'-CTCACCACCAACTTCATCCACGTTCACATA	3	211
IVSII-654 M (C-T)	5'-GAATAACAGTGATAATTTCTGGGTTAACGT	2	830
Hb-E Cd 26 (G-A)(M)	5'-TAACCTTGATACCAACCTGCCCAGGGCGTT	3	267
African Mutations			
-28 M (A-G)	5'-AGGGAGGGCAGGAGCCAGGGCTGGGCTTAG	4	624
-88 M (C-T)	5'-TCACTTAGACCTCACCCTGTGGAGCCTCAT	4	655
C 24 M (T-A)	5'-TGGGGCAAGGTGAACGTGGATGAAGTTAGA	4	472
Hb-S-M	5'-CCCACAGGGCAG TAACGGCAGACTTCTGCA	3	208
Hb-S-N	5'-CCCACAGGGCAGTAACGGCAGACTTCTGCT	3	208



6. ARMS primers for some mutations are complementary to the reverse strand and these are used with another "common primer" complementary to the forward strand (primer 4).
7. In each reaction two additional primers (1 & 2) are used to amplify 861bp fragment of the distal end of β-globin gene. This works as an internal control in the ARMS PCR. The location of the primers 1 & 2 is such that it also allows identification of 619bp deletion in the β-globin gene. If the del 619bp mutation is present the control fragment, instead of the usual 861bp, is reduced to 242bp.

Multiplex ARMS

1. A multiplex ARMS PCR for 12 β-thalassaemia mutations found in the Pakistani population is described. Similar multiplexes for the other ethnic populations can also be developed.
2. Three combinations of primers for multiplex ARMS of β-thalassaemia are shown in Table 13.2. The multiplexes AD-1 and AD-2 contain the ARMS primers used with "common primer 3" while the AD-3 contains the ARMS primers that are used with "common primer 4".
3. The amplified products of all the mutations tested in each multiplex are sufficiently different for resolution by mini polyacrylamide gel electrophoresis. However, the size of the fragments generated by IVSI-1 and IVSI-5 differ by only 5bp and are difficult to resolve on a mini gel. Similarly there is no difference between the fragments of Cd30 (G-C), Cd30 (G-A) and IVSI-1 mutations. The problem of differentiating IVSI-1 and IVSI-5 is overcome by adding IVSI-1 primer to AD-1 and AD-2 multiplexes. IVSI-5 results in amplification with AD-1, but IVSI-1 causes amplification with AD-1 and AD-2. Amplification with AD-2 but not AD-1 indicates Cd30. The difference between Cd30 (G-C) and Cd30 (G-A) is only of academic interest because the same normal primer is used to differentiate between the homozygotes and heterozygotes of the two mutations. The AD-3 primer combination includes Cd15 and Cap+1 and both of these primers are used with "common primer 4".
4. An allelic ladder for the respective mutations is prepared by pooling the PCR products of separately amplified reactions of various mutations. The allelic ladder is kept frozen in aliquots. 5µl of the pooled product is used in all polyacrylamide gel electrophoresis runs.
5. The amplified products of the multiplex ARMS and the respective allelic ladders are run on 6% polyacrylamide mini gels.
6. Interpretation of the multiplex ARMS results is shown in Fig. 13.1.



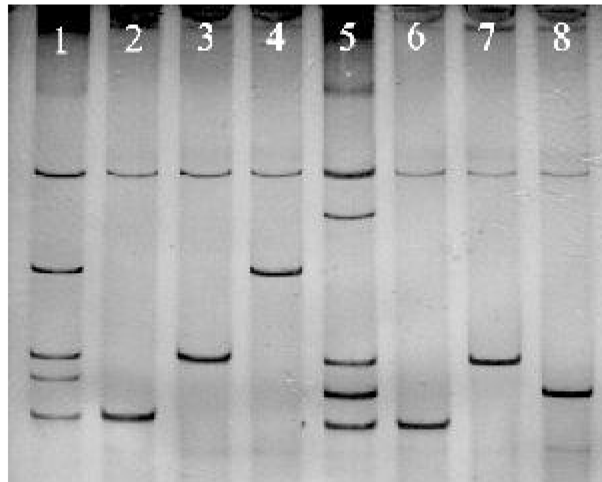


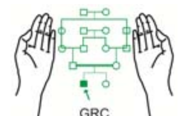
Fig. 13.1. Silver stained mini polyacrylamide gel electrophoresis of multiplex ARMS PCR products. Lane 1 and 5 show allelic ladders for the AD-1 and AD-2 multiplexes. All lanes show the 861bp internal control fragment. The sample in lane 2, 3 and 4 are positive for Fr 8-9 (+G), IVSI-5 (G-C) and Fr 41-42 (-TTCT) mutations respectively. Lanes 6, 7 and 8 show Cd5 (-CT), IVSI-1 (G-T) and Fr 16 (-C) mutations respectively.

Table 13.2. Multiplex ARMS primer combinations for β-thalassaemia mutations found in the Pakistani population.

Primer ID:	**Mutations:**	**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

Homozygous or heterozygous mutation

1. Once the mutation is identified the next step is to find whether the mutation is homozygous or heterozygous. This is done by setting up a separate PCR reaction in which the normal allele of the respective mutation is tested. The list and the sequences of the normal ARMS primers are shown in Table 13.1.



2. In a homozygote either two copies of the same mutation (true homozygote) or two different mutations (compound heterozygote) are seen.
3. The true homozygote shows only one mutation. Its homozygosity is ascertained by testing for the normal allele checked in a separate PCR reaction. The compound heterozygote shows two different mutations.
4. A heterozygote has the mutant as well as the normal allele. There is no need to test for the normal allele if the individual is known to have thalassaemia trait.

Sequencing of β-globin gene

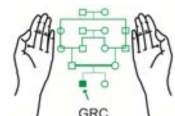
The β-globin gene sequencing is done to identify an unknown mutation or to confirm the results of ARMS PCR. The gene is approximately 1.5 kb in length. On the 36cm capillary of genetic analyzer approximately 600 bases can be sequenced in one go. The entire β-globin gene can be sequenced in overlapping segments.

Sequencing strategy

Most of the β-thalasaemia mutations are located in the first two exons and the intervening sequence (segment-I). The segment-II mostly covers the IVS-II which is an unusual site for the mutations. The segment-III covers the third exon and the terminal portion of the gene. It also contains only a few uncommon mutations.

- Segment-I
  - Target region: Promoter region & Exon-I
  - Forward primer: 5'-AGACATAATTTATTAGCATGCATG
  - Reverse primer: 5'-CTCCTTAAACCTGTCTTGTAACCT
  - Fragment size: 809 bp
  - Sequencing primer: 5'-AGACATAATTTATTAGCATGCATG
- Segment-II
  - Target region: Exon-I, IVS-I & Exon-II
  - Forward primer: 5'-ACCTCACCCTGTGGAGCCAC
  - Reverse primer: 5'-CCCCTTCCTATGACATGAACTTAA
  - Fragment size: 676 bp
  - Sequencing primer: 5'-ACCTCACCCTGTGGAGCCAC
- Segment-III
  - Target region: Exon-III & Poly-A site
  - Forward primer: 5'-CAATGTATCATGCCTCTTTGCACC
  - Reverse primer: 5'-GAGTCAAGGCTGAGAGATGCAGGA
  - Amplified fragment: 861 bp
  - Sequencing primer: 5'-CAATGTATCATGCCTCTTTGCACC

The details of the sequencing protocol are given in Chapter 10.



Results:

The results of normal sequence, IVSI-5 (G-C) and Fr 8-9 (+G) mutation are shown in Fig 13.2 and 13.3.

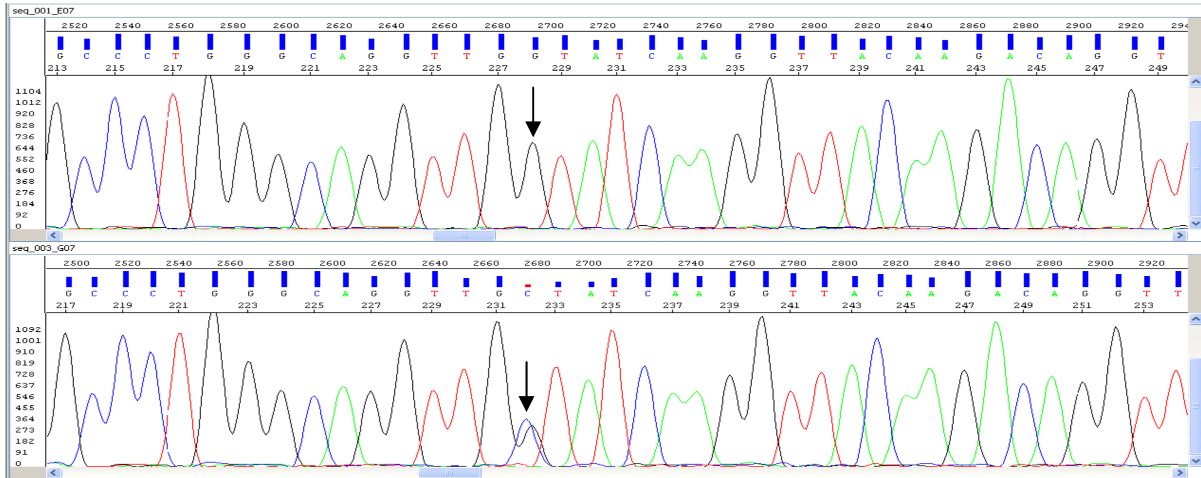


Fig 13.2. Electropherogram of the β-globin gene showing the sequence around the first exon-intron junction. The upper half shows the normal sequence whereas IVSI-5 (G-C) substitution (arrow) can be seen as overlapping peaks (black and blue) in the lower part of the picture.

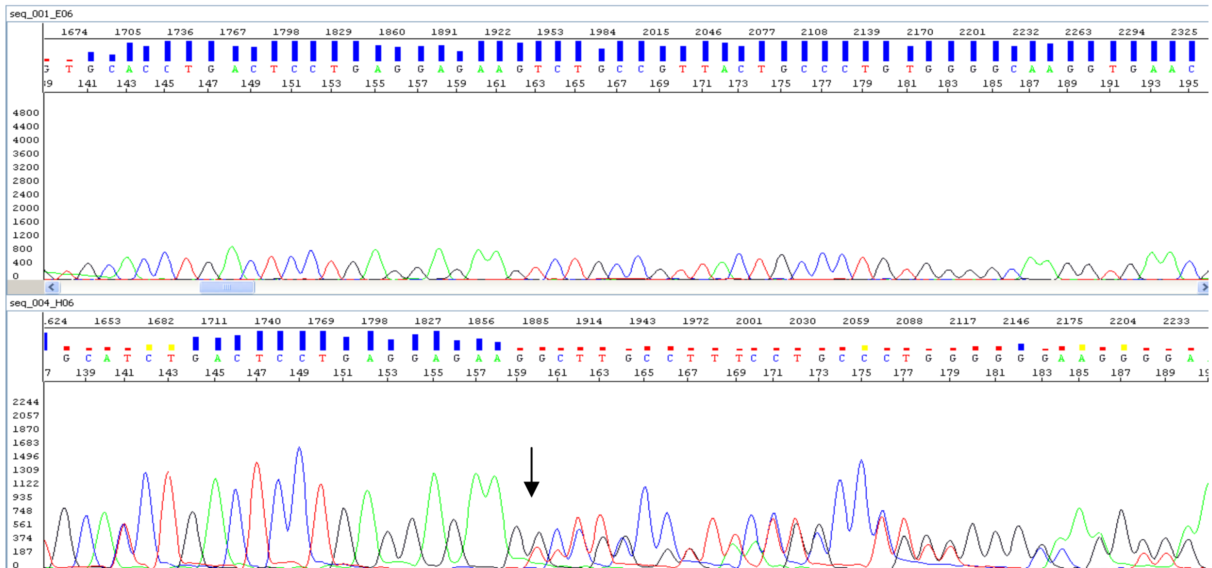
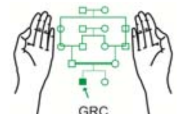


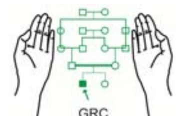
Fig 13.3. Upper half of the picture shows normal sequence of β-globin gene whereas Fr 8-9 (+G) insertion is seen to cause complete disruption of the sequence beyond codon eight (arrow) in the lower part of the picture.



Prenatal Diagnosis (PND)

PND by direct mutation analysis

1. Identify the parent's mutations by testing the DNA of each parent or an affected child if any of the parents is not available.
2. Carefully dissect the chorionic villus sample (CVS) to remove any maternal decidua.
3. Extract DNA from the CVS (fetus) (Chapter 2).
4. Setup ARMS PCR as follows (also see chapter 7):
    1. If both parents have the same mutation label ten tubes and add:
        i. Tubes1-5: primers 1+2 + common primer + mutation primer.
        ii. Tubes 6-10: primers 1+2 + common primer + normal primer for the mutation.
        iii. In tubes 1-5 add DNA of father, mother, CVS (in duplicate), and negative control for the mutation.
        iv. In tubes 6-10 add DNA of CVS (in duplicate), positive control for normal allele (normal DNA), negative control for normal allele (homozygous for the mutation) and no DNA (reagent blank).
        v. Gel electrophoresis and the interpretation of the results are shown in Fig. 13.4.
    2. If the two parents have different mutations label nine tubes and add:
        i. Tubes1-4: primers 1+2 + common primer + primer of father's mutation
        ii. Tubes 5-9: primers 1+2 + common primer + primer of mother's mutation
        iii. In tubes 1-4 add DNA of father, CVS (in duplicate) and negative control for father's mutation.
        iv. In tubes 5-9 add DNA of mother, CVS (in duplicate), negative control for mother's mutation and no DNA (reagent blank).



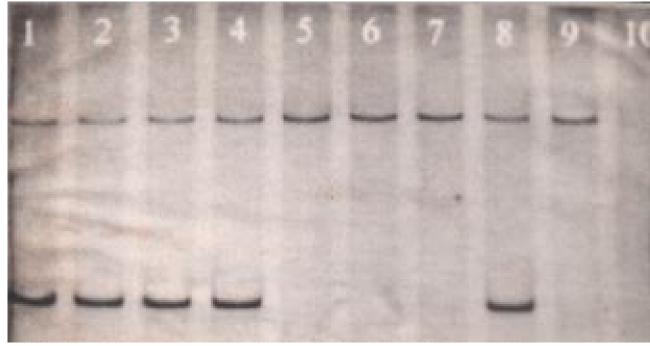
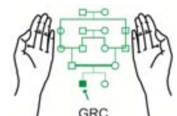


Fig. 13.4. Polyacrylamide gel electrophoresis of prenatal diagnosis for β-thalassaemia. All lanes except lane 10 show 861bp internal control bands. Absence of any result in the reagent blank (Lane 10) excludes the false positive results. Lanes 1 & 2 shows the parent's mutations (IVSI-5). Lanes 3 & 4 show the same mutation (IVSI-5) tested in duplicate in the fetal DNA. Lane 5 is a negative control for IVSI-5 mutation. Lanes 6 & 7 shows testing for the normal allele of IVSI-5. Its absence in the fetal DNA confirms that the fetus has homozygous IVSI-5. Lanes 7 and 8 are negative and positive controls for the normal allele of IVSI-5 respectively. Lane 8 is a negative control for the IVSI-5 allele.

5. **Exclusion of maternal contamination**
    1. Presence of maternal tissue in the CVS can be a potential source of error in prenatal diagnosis. Since the mother is a carrier who has the mutant as well as the normal alleles, contamination of CVS by the maternal tissue would make all normal or homozygous fetal results to appear as thalassaemia trait.
    2. When the parents have the same mutation and the fetal diagnosis is "normal" or "homozygous (thalassaemia major)" maternal contamination is ruled out.
    3. When the parents have two different mutations and the mother's mutation is not present in the CVS, maternal contamination is ruled out.
    4. When the parents have the same mutation and the fetal diagnosis is "heterozygous (thalassaemia trait)" maternal contamination can not be ruled out.
    5. Experience has shown that meticulous cleaning of the CVS is enough to safeguard against any errors due to maternal contamination.
    6. The maternal contamination in the CVS can be tested by short tandem repeat (STR) analysis.
    7. The CVS and the maternal DNA are run for various STR loci (Chapter 9 & 15).



8. The STR marker is called “informative” when its alleles can distinguish between the maternal and the fetal DNA. For example at D21S11 locus if the mother has alleles 28,29 and the fetus (CVS) has 28,31. The marker is informative because allele 29 and 31 are exclusive for the mother and the fetus respectively. It also indicates that there is no maternal contamination. It would have indicated maternal contamination had the exclusive maternal allele (29) also been seen in the fetus which is usually expressed as 28,31(29).
9. STR locus D21S11 is often used because of its high degree of polymorphism. It’s limitation is that if the fetus has trisomy 21 and the extra chromosome 21 is of maternal origin the result could appear as maternal contamination.
10. If the SRT is not informative at any locus the analysis is done at other loci.

PND of a genetic disorder by linkage analysis

In couples when the parent’s mutation is not identified PND can also be done by linkage analysis. There are several single nucleotide polymorphisms (SNP) and short tandem repeats (STR) that are closely linked to several important genetic disorders. The polymorphic loci are inherited en-block with the gene of interest and can be used to track their inheritance.

Linkage analysis by SNP

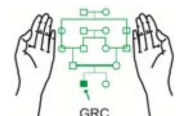
The SNPs are recognized by the strategic use of restriction enzymes. In the first step DNA of the parents and the affected child are tested to establish linkage of the SNP with the chromosome carrying the mutation. Once an informative marker is identified the fetal DNA is tested to determine its genotype. By convention the SNP is written as “+” when it is present and as “-“ when it is not present (Chapter 8).

Pre-requisites of linkage based prenatal diagnosis:

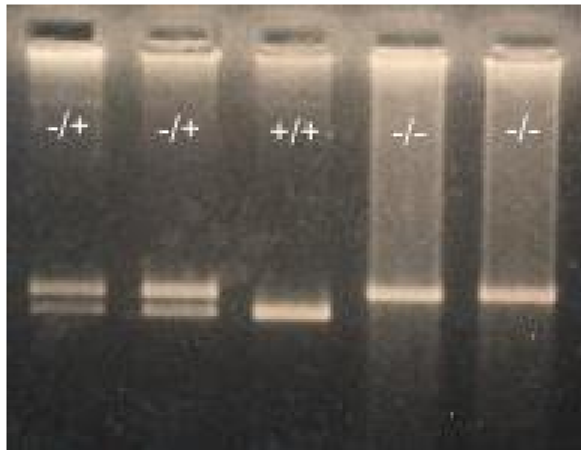
1. The couple must have a previously affected child.
2. At least one fully informative marker should be available. The marker is called informative when both of the parents are heterozygous (-/+).
3. Linkage based prenatal diagnosis is not possible if any of the parents is homozygous (+/+) for the marker.
4. Linkage based prenatal diagnosis may be possible by using more than one partially informative markers (when only one of the parents is heterozygous for the marker).
5. The linkage based diagnosis may turn out to be incorrect if during the meiotic cross over marker and the gene of interest dissociate (separate) from each other.

Example:

β-globin gene complex contains at least seven SNPs that can be used to carry out linkage based prenatal diagnosis of thalassaemia (Varawalla et al, 1992). One of the SNPs can be recognized by restriction enzyme Hinc-II. The results of genotyping at this SNP in a couple with an affected child are explained in Fig. 13.5.



(A)



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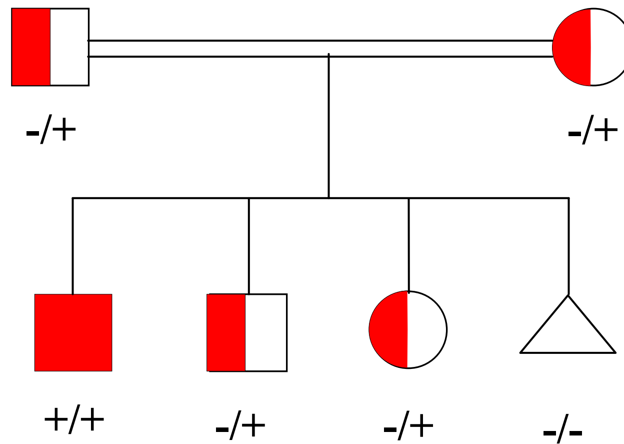
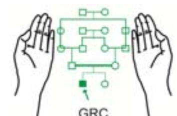


Fig. 13.5. (A) Ethidium bromide stained agarose gel electrophoresis of Hinc-II digested fragments of β-globin gene. Father and the mother have -/+ genotype. The affected child of the couple has +/+ genotype indicating that the chromosome carrying the + site in the father and the mother has the β-thalassaemia mutation. The fetal DNA has -/- genotype indicating not affected by β-thalassaemia.

Linkage analysis by STR

Linkage analysis may also be done by STR markers. Several important STR loci are known to be present in many important genes. Duchene muscular dystrophy (DMD) is a good example in which at least four di-nucleotide STRs present in the Introns 44, 45, 49 and 50 of the dystrophin gene on X-chromosome can be used. The intra-genic STRs have an additional advantage as their failure to amplify indicates deletions and inversions in the dystrophin gene (Fig. 13.6).



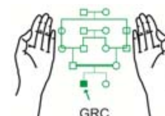
Example

Primers for amplification of di-nucleotide STRs in the four introns of dystrophin gene.

DMD-44 F	5’-TCCAACATTGGAAATCACATTTCAA
DMD-44 R	5’-TCATCACAAATAGATGTTTCACAG
DMD-45 F	5’-GAGGCTATAATTCTTTAACTTTGGC
DMD-45 R	5’-CTCTTTCCCTCTTTATTCATGTTAC
DMD-49 F	5’-CGTTTACCAGCTCAAAATCTCAAC
DMD-49 R	5’-CATATGATACGATTCGTGTTTTGC
DMD-50 F	5’-AAGGTTCCTCCAGTAACAGATTTGG
DMD-50 R	5’-TATGCTACATAGTATGTCCTCAGAC

Each pair of primer for the respective intron is used for amplification in a separate tube as follows:

- Reaction volume: 25 µl
- Primer concentration: 1 µl (5 pmol each/µl)
- Taq polymerase: 0.5 units (0.1µl)
- Template DNA: 1µl (200ng)
- Thermal cycling:
    - Initial denaturation: 1minute at 94°C
    - No. of cycles: 25
      Denaturation: 30 seconds at 94°C
      Annealing: 30 seconds at 62°C
      Extension: 2 minutes at 65°C
      Final extension: 3 minute at 72°C
- Electrophoresis: 16 X 20 X 0.1cm 6% polyacrylamide gel.
  Load 3µl amplified product in 3µl loading dye
  Run at 100 volts overnight.
- Staining: 0.1% Silver nitrate.
- Result: Fig 13.6



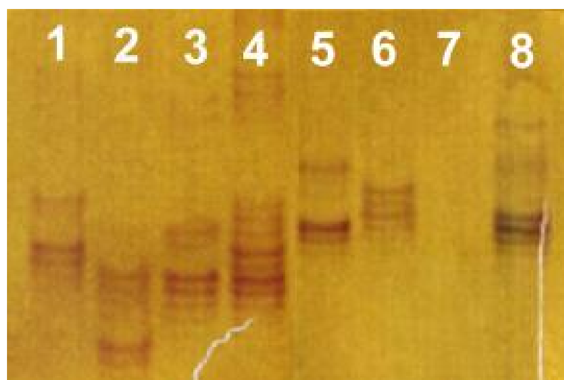


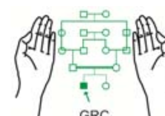
Fig. 13. 6. PAGE after PCR amplification of di-nucleotide repeats in Intron 44 (Lanes 1-4) and Intron 45 (Lanes 5-8) of dystrophin gene in a family with DMD. Lanes 1 & 5 show father's sample, lanes 2 & 6 show mother's sample, lanes 3 & 7 show sample of the child affected by DMD, and lanes 4 & 8 show sample of fetal DNA. Sample of the affected child in lane 3 shows amplification product whereas the same sample in lane 7 does not show any amplification indicating a deletion in the dystrophin gene involving intron 45. The fetal DNA sample (lanes 4 & 8) does not show the deletion in intron 45. The result of Intron 44 can also be used for linkage analysis. The father (lane 1) has one allele due to one X chromosome (a faint band of PCR stutter product can also be seen). The mother (lane 2) shows two alleles due to two X chromosomes. The affected child (lane 3) being a male has one allele that is inherited from the mother. This indicates that out of the mother's two alleles the larger allele corresponding to that of the affected child can be used as a marker for the abnormal X chromosome. The fetal sample (lane 4) shows two alleles one inherited from the father and the other from the mother. The result is consistent with female fetus carrier for DMD.

Limitations of linkage based PND

1. The couple should have a living affected child.
2. At least one informative marker must be present.
3. There is approximately 1% chance that during meiotic cross-over the marker and the abnormal gene could dissociate causing error in diagnosis.
4. Presence of mutation out-side the abnormal gene can cause error in diagnosis.

Sources of error in PND

1. Maternal contamination in fetal sample
2. PCR failure
3. Clerical mistakes
4. Meiotic crossover in linkage analysis
5. Non paternity



Screening for Trisomies

Chromosomal trisomies can be detected by STR analysis. Trisomies 13, 18 and 21 constitute over 90% of the clinically significant problems. The three trisomies can be quickly screened by PCR amplification of STR loci. The principle of screening is that each chromosome carries one allele of an STR. In a trisomy three instead of the usual two alleles are seen. A technical problem often arises if two or all three of the alleles on the three chromosomes have the same number of repeat units. The problem is solved either by testing additional loci or by densitometry or automated STR analysis on genetic analyzer (Chapter 9).

The following allele patterns may be seen at an STR locus (example D21S11):

1. Normal
    a. Disomic di-allelic (28,29)
    b. Disomic mono-allelic (28,28)
2. Abnormal
    a. Trisomic tri-allelic (28,29,30)
    b. Trisomic di-allelic (28,29,29)
    c. Trisomic monoallelic (28,28,28)

The STR allele sizing is done by PCR amplification of the locus followed by gel electrophoresis. In manual allele sizing gel electrophoresis is done with an allelic ladder. The results may be interpreted by visual inspection of the gel or by densitometry. The trisomy screening can be done without allele sizing if the amplified DNA samples of the father, mother and the subject are run simultaneously on a polyacrylamide gel (Fig. 13.7) The DNA amplified with a fluorescent labeled primer when run on genetic analyzer also provides information on the allele sizes (Fig. 13.8).

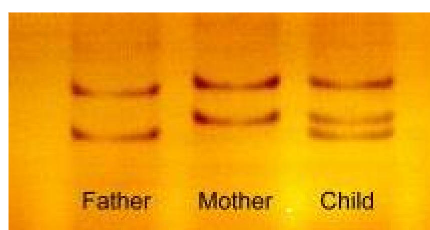
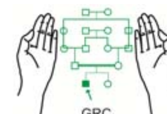


Fig. 13.7. Polyacrylamide gel electrophoresis of PCR amplification at D21S11 locus in a couple suspected to have child affected by trisomy 21. The father and the mother have a disomic diallelic pattern where as the child shows trisomic triallelic pattern confirming trisomy 21. In addition it is also visible that the child inherited the extra chromosome 21 from the mother.



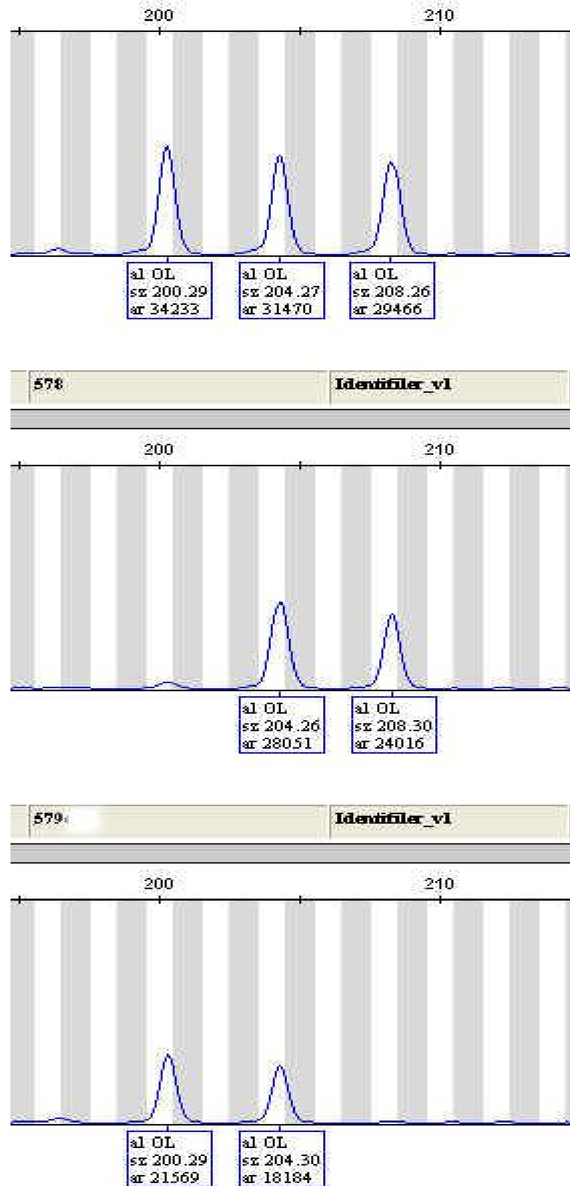
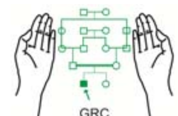


Fig. 13.8. PCR amplification at D21S11 locus ran on a genetic analyzer. The father and the mother have a disomic diallelic pattern whereas the affected child (top) shows trisomic triallelic pattern confirming trisomy 21.

Primers used for screening of trisomies:

<u>Trisomy 13:</u>

D13S317-F	5'-ACAGAAGTCTGGGATGTGGA
D13S317-R	5'-GCCCAAAAAGACAGACAGAA
D13S634-F	5'-TCCAGATAGGCAGATGATTCAAT
D13S634-R	5'-CCTTCTTCTTCCCATTGATA



Trisomy 18

D18S51-F	5'-CAAACCCGACTACCAGCAAC
D18S51-R	5'-GAGCCATGTTCATGCCACTG
D18S535-F	5’-TCATGTGACAAAAGCCACAC
D18S535-R	5’-AGACAGAAATATAGATGAGAATGCA

Trisomy 21

D21S11-F	5’-GTGAGTCAATTCCCCAAG
D21S11-R	5’-GTTGTATTAGTCAATGTTCTCC
D21S1411-F	5’-ATGATGAATGCATAGATGGATG
D21S1411-R	5’-AATGTGTGTCCTTCCAGGC
D21S1412-F	5’-CGGAGGTTGCAGTGAGTT
D21S1412-R	5’-GGGAAGGCTATGGAGGAGA
D21S1414-F	5’-AAATTAGTGTCTGGCACCCAGTA
D21S1414-R	5’-CAATTCCCCAAGTGAATTGCCTTC

Limitations

Detection of trisomies by PCR is only a screening method and it is not a substitute for cytogenetic analysis. Its major limitation is that in PCR the smaller alleles may be preferentially amplified giving the false impression of trisomy.

Bibliography

1. Old JM, Varawalla NY, Weatherall DJ (1990) Rapid detection and prenatal diagnosis of β-thalassaemia: studies in Indian and Cypriot populations in the UK. Lancet 336: 834-837.
2. Clemens PR, Fenwick RG, Chamberlain JS, Gibbs RA, Andrade M, Chakraborty R (1991) Carrier detection and prenatal diagnosis in Duchenne and Becker muscular dystrophy families, using dinucleotide repeat polymorphisms. Am J Hum Genet 49: 951-960.
3. Ahmed S, Petrou M, Saleem M (1996) Molecular genetics of β-thalassaemia in Pakistan: a basis for prenatal diagnosis. Br J Haematol 94: 476-481.
4. Old JM (1996) Haemoglobinopathies. Prenat Diagn 16: 1181-1186.
5. Adinolfi M, Pertl B, Sherlock J (1997) Rapid detection of aneuploidies by microsatellites and the quantitative fluorescent polymerase chain reaction. Prenat Diagn 17: 1299-1311.
6. Huisman TJH, Carver MFH (1998) The δ- and β-thalassaemia repository. Haemoglobin 22: 169-195.
7. Ahmed S, Saleem M, Petrou M, Sultana N, Raashid Y, Waqar A, Anwar M, Karamat KA (2000) Prenatal diagnosis of β-thalassaemia in Pakistan: experience in a Muslim country. Prenat Diagn 20: 378-383.