Reliability of prenatal diagnosis of genetic diseases by analysis of amplified trophoblast DNA

M C Rosatelli, R Sardu, T Tuveri, M T Scalas, A Di Tucci, M De Murtas, G Loudianos, G Monni, A Cao

Abstract
Dot blot analysis on enzymatically amplified trophoblast DNA with allele specific oligonucleotide probes is currently used for the prenatal diagnosis of single gene disorders characterised at the molecular level, such as the β thalassaemias, phenylketonuria, sickle cell anaemia, and α-antitrypsin deficiency. A potential problem with the use of this procedure is the co-amplification of maternal sequences, which may obscure the diagnosis in the fetus. To address this question, we carried out prenatal diagnosis of β thalassaemia in 300 couples at risk by dot blot analysis on enzymatically amplified DNA with 32P or horseradish peroxidase labelled allele specific oligonucleotide probes. We verified the diagnosis obtained by this procedure with oligonucleotide hybridisation on electrophoretically separated non-amplified trophoblast DNA fragments. We detected no co-amplified maternal sequences, even with a faint signal, in the dot blot of trophoblast DNA from those fetuses diagnosed as normal or homozygotes, nor in those diagnosed as heterozygotes, who were born to parents carrying different mutations and had inherited the paternal mutation. These results indicate that, when careful dissection of trophoblast tissue from maternal decidua is carried out, amplification of chorionic villi DNA is not associated with amplification of maternal DNA sequences. We may thus conclude that dot blot analysis of trophoblast DNA is a very reliable procedure for prenatal diagnosis.

Nowadays a number of mendelian disorders can be detected in fetal trophoblasts by using enzymatically amplified DNA.1-4 Those disorders caused by a gross structural rearrangement of the DNA or those affecting a restriction recognition site are directly detected by visualisation with silver nitrate or ethidium bromide staining of the discrete bands resulting from gel electrophoresis of restriction enzyme digested enzymatically amplified DNA.4-6 This method is very simple because it circumvents the use of molecular probes. Disorders produced by point mutations or minimal rearrangements of DNA, such as the deletion or addition of a few nucleotides, are defined by dot blot analysis on amplified DNA with allele specific oligonucleotide probes.7 The sensitivity of this procedure allows the use of non-radioactive probes, such as horseradish peroxidase labelled oligonucleotides.8 The main advantages of these procedures are the sensitivity (less than 0·05 μg of fetal DNA is necessary) and the rapidity (the results can be obtained within 24 hours of sampling). A potential problem, however, may arise from the co-amplification of residual maternal decidua cells which may confuse the diagnosis of the fetus.

In this paper, we report the results of prenatal diagnosis of β thalassaemia in 300 pregnancies at risk carried out by dot blot analysis on enzymatically amplified trophoblast DNA with 32P8 or horseradish labelled oligonucleotide probes,9 and verified by oligonucleotide hybridisation on non-amplified trophoblast DNA fragments separated by gel electrophoresis.10

In none of the cases investigated was amplification of maternal sequences found and diagnoses on amplified DNA were confirmed without exception by the analysis of non-amplified DNA. This indicates that analysis of amplified DNA is a reliable procedure for fetal diagnosis of genetic diseases.

Patients and methods
Three hundred pregnant women of Italian descent requesting prenatal diagnosis because they were at risk for thalassaemia major were included in this study.
Definition of the β thalassaemia mutation in both parents was obtained by dot blot analysis on enzymatically amplified DNA with a series of allele specific oligonucleotide probes complementary to the eight most common mutations in the Mediterranean area. These were the G→A substitution at IVS–1 position 110 (β+ IVS–1 nt 110), the codon 39 nonsense mutation (β39N), the T→C nucleotide substitution at IVS–1 position 6 (β+ IVS–1 nt 6), the G→A transition at IVS–1 position 1 (β+ IVS–1 nt 1), the G→A substitution at IVS–2 position 1 (β+ IVS–2 nt 1), the frameshift mutation (GAG→GG) at codon 6 (β6–1 bp), the C→G substitution at position 87 from the CAP site (β+ −87), and the C→G mutation at IVS–2 position 745 (β+ IVS–2 nt 745).11

Chorionic villi sampling was carried out transabdominally from 10 to 14 weeks' gestation. Trophoblast tissue was accurately dissected from maternal decidua under an inverted microscope. Fetal diagnosis was accomplished by dot blot analysis on enzymatically amplified trophoblast DNA with oligonucleotide probe(s) complementary to the mutation(s) detected in the parents. All the diagnoses were verified by oligonucleotide hybridisation on trophoblast DNA restriction fragments separated by agarose gel electrophoresis. DNA amplification, dot blot analysis, and oligonucleotide analysis on electroforetically separated DNA fragments were carried out as previously described.7,10,12,13 The oligonucleotide probes were labelled either with 32P or horse radish peroxidase (a generous gift from Cetus Corporation, CA).

Results
The β globin genotypes of the 300 couples at risk are reported in table 1.
Out of 300 fetuses investigated, 80 were normal and there were 69 homozygotes, 19 compound heterozygotes, and 132 heterozygotes for β thalassaemia (table 2). Figs 1 and 2 show a representative dot blot of the DNA from normal, homozygous, and heterozygous fetuses and their parents. We looked for the presence of co-amplified maternal sequences, even with a faint signal, in the dot blots of trophoblast DNA.

| Table 1 β thalassaemia mutations detected in the couples at risk investigated. |
|------------------|--------|------|
| β thalassaemia mutation | No | % |
| β39N | 225 | 75 |
| β39N+ IVS–1 nt 110 | 16 | 5 |
| β39N+ IVS–1 nt 6 | 8 | 3 |
| β39N/others* | 28 | |
| β+ IVS–1 nt 110/β+ IVS–1 nt 110 | 5 | |
| β+ IVS–1 nt 110/β+ IVS–1 nt 6 | 4 | |
| β+ IVS–1 nt 110/other | 6 | |
| β+ IVS–1 nt 6/other | 4 | |
| β39N–2 nt 1/β39N–2 nt 1 | 1 | |
| β39N–1 nt 1/β39N–1 nt 1 | 1 | |
| [6]–1 bp/[6]–1 bp | 1 | |
| Others* | 1 | |
| Total | 300 | |

* = [6]–1 bp, β39N–2 nt 1, β+ IVS–2 nt 745, δβ thal, Hb Lepore, Hb S.

Table 2 Overall results of prenatal diagnosis of β thalassaemia by dot blot analysis on amplified DNA.

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<table>
<thead>
<tr>
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<tbody>
<tr>
<td>Pregnancies monitored</td>
<td>300</td>
<td></td>
</tr>
<tr>
<td>Homozygous fetuses</td>
<td>88</td>
<td></td>
</tr>
<tr>
<td>Heterozygous fetuses</td>
<td>132</td>
<td></td>
</tr>
<tr>
<td>Normal fetuses</td>
<td>80</td>
<td></td>
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<tr>
<td>Failures</td>
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<tr>
<td>Misdiagnoses†</td>
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*Of whom 19 were compound heterozygotes for two different mutations.
†All the diagnoses were confirmed by oligonucleotide analysis on non-amplified DNA.

Figure 1 Dot blot analysis on amplified DNA with 32P labelled allele specific oligonucleotide probes. β110, β1–6, and β39 oligonucleotide probes complementary to the β+ IVS–1 nt 110, β+ IVS–1 nt 6, and β39 mutations, respectively. βN110 and βN39= oligonucleotide probes complementary to normal DNA sequences at a position corresponding to each of the β thal mutations investigated. N and βTH= normal and homozygous affected control. Fa, Mo, and Fe= indicate father, mother, and fetus. (a) Normal fetus showing no hybridisation to the oligonucleotide probe complementary to the β+ IVS–1 nt 110 mutation. (b) Heterozygous fetus showing no hybridisation to the oligonucleotide probe complementary to the mother's mutation (β1–6). (c) Homozygous affected fetus showing no hybridisation to the oligonucleotide probe complementary to normal DNA sequences corresponding to the β39 mutation.
DNA from fetuses diagnosed as normal or homozygous, as well as those diagnosed as heterozygous who were born to parents carrying different mutations and who had inherited the paternal allele. In the normal fetuses, we did not ever detect a positive hybridisation to an oligonucleotide probe complementary to the mutation detected in the mother. Similarly, in the homozygous fetuses, hybridisation to oligonucleotide probes complementary to the normal sequences at the position corresponding to the mutation detected in the mother was never observed.

In 19 heterozygous fetuses who were born to parents carrying different mutations and who had inherited the paternal mutation, we never observed a positive hybridisation to an oligonucleotide probe complementary to the maternal mutation.

In all cases tested, diagnosis obtained by dot blot analysis on amplified DNA was confirmed by oligonucleotide hybridisation on non-amplified DNA.

Out of 300 fetuses examined, 68 went to term and diagnosis was confirmed by DNA analysis.

Discussion

This paper, based on a large number of cases, indicates that prenatal diagnosis of β thalassaemia by dot blot analysis on enzymatically amplified DNA with allelic specific oligonucleotide probes is a very reliable diagnostic procedure. We did not detect any co-amplified maternal sequences, even with a faint signal, in the dot blot of trophoblast DNA from those fetuses diagnosed as normal or homozygous, or those diagnosed as heterozygous who were born to parents carrying different mutations and who had inherited the paternal mutation.

Furthermore, the results obtained on amplified DNA were confirmed, without exception, by the analysis of non-amplified DNA. We can therefore conclude that the potential problems in prenatal diagnosis using amplification of maternal sequences do not occur when careful dissection of trophoblast tissue from maternal decidua is carried out. Since this study was completed, we have introduced this procedure for routine use in the laboratory of our unit for prenatal diagnosis of genetic diseases. The results obtained in this study are obviously also relevant to other single gene disorders characterised at the molecular level, such as α1-antitrypsin deficiency, cystic fibrosis, and phenylketonuria which could be diagnosed in the fetus by dot blot analysis on amplified DNA.

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5 Chehab FF, Cai SP, Doherty M, Kan YW, Cooper S, Rubin EM. Simple and rapid nonradioactive DNA analysis for the prenatal diagnosis of sickle cell anaemia and homozygous α-thalassaemia. Am J Hum Genet 1987;41 (suppl 3):269A.

![Figure 2. Dot blot analysis on amplified DNA with horseradish peroxidase labelled oligonucleotide probes. βN and βTH = oligonucleotide probes complementary to normal and mutated (β<sup>TH</sup>) DNA sequences respectively. Fa, Mo, Fe = indicate father, mother, and fetus. Homozygous affected fetus showing no hybridisation to an oligonucleotide probe complementary to normal DNA sequences corresponding to the mutation.](http://jmg.bmj.com/Downloadedfromgroup.bmj.com)
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