Prenatal diagnosis of β thalassaemia by oligonucleotide analysis in Mediterranean populations

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SUMMARY We have used four oligonucleotide probes and two restriction enzymes to detect the β thalassaemia mutation in a group of 61 couples of Italian descent who were prospective parents. We have been able to define the β thalassaemia mutation in both parents in 47 couples and in only one parent in 12 couples. Prenatal diagnosis was accomplished successfully either by amniocyte (two) or trophoblast (26) DNA analysis in 28 couples in which the pregnancy was in progress. These results indicate that direct identification of the mutation by oligonucleotide or restriction endonuclease analysis is a practical and useful method for prenatal diagnosis of β thalassaemia in childless couples.

Prenatal diagnosis of β thalassaemia can at present be accomplished in the great majority of cases by amniocyte or trophoblast DNA analysis.¹⁻³ Two different procedures are used: (1) the direct identification of the mutation by oligonucleotide hybridisation or restriction endonuclease analysis, and (2) linkage analysis with polymorphic restriction enzyme sites in the β globin gene cluster. A critical prerequisite for linkage analysis is the knowledge of linkage phase, which allows distinction of the normal from the β thalassaemia chromosome in each parent. This can be accomplished easily in couples with a previous normal or affected child; in childless couples, however, polymorphism analysis entails a cumbersome and sometimes futile family study, and is thus not applicable to large scale programmes. Direct detection of the mutation, on the other hand, requires information on the type and prevalence of β thalassaemia mutations in each high risk population in order to screen prospective parents with the appropriate probes. In the last few years, several studies have shown that in each population at risk a limited number (from two to four) of β thalassaemia mutations are prevalent.⁴⁻¹⁵ In each population a few oligonucleotide probes or restriction enzymes may thus allow prenatal diagnosis in the great majority of cases.

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In this study, we have used four oligonucleotide probes and two restriction enzymes to detect the β thalassaemia mutation directly in a group of 61 couples of Italian descent at risk for β thalassaemia who were prospective parents. We have been able to define the β thalassaemia mutation in both parents in 47 couples and in only one parent in 12 out of 61 couples investigated. Prenatal diagnosis has been accomplished successfully so far either by amniocyte or trophoblast DNA analysis in 28 couples in which a pregnancy was in progress. These results indicate that direct identification of the mutation by oligonucleotide or restriction endonuclease analysis is a practical and useful method of prenatal diagnosis of β thalassaemia in childless couples.

Patients

Sixty one childless couples at risk for β thalassaemia, who originated from different high risk areas of Italy (Calabria, Sicilia, Campania, Puglia), presented at our Genetic Unit for counselling. All prospective parents but two, heterozygotes for Hb Lepore or $\delta\beta$ thalassaemia, were carriers of high A_2 β thalassaemia. Pregnancy was in progress in 33 cases. In the counselling session the relative merits of amniocentesis and chorionic villus sampling were extensively discussed. Four couples were monitored by amniocentesis and 27 by trophoblast sampling.

Methods

Trophoblast sampling was carried out with biopsy forceps according to Kaplan *et al.*¹⁶ Oligonucleotide and restriction endonuclease analysis was performed using previously described methods.^{17 18}

DNA from prospective parents was screened with four oligonucleotide probes complementary to the most frequent mutations in Italians, namely the G–A substitution at position 110 of the first intervening sequence (IVS–1)(β^+ IVS–1 nt 110),¹⁹ the C–T substitution at codon 39 (β^{39}),²⁰ the T–C substitution at position 6 of IVS–1 (β^+ IVS–1 nt 6),⁴ and the G–A substitution at position 1 of IVS–1 (β^0 IVS–1 nt 1).⁴ The sequence of the oligonucleotide probes to be used was defined on the basis of the relative frequency of these four mutations in the high risk areas from which the parents originated.¹⁴ For each of the four β thalassaemia mutations investigated, two sets of oligonucleotide probes were used, one complementary to the β globin gene sequence around the mutation and one homologous to the normal β globin gene sequences at the same position.

The DNA was cleaved with *Bam*HI and the 1.8 kb *Bam*HI fragment in which all these four mutations reside was separated from bulk DNA by agarose gel electrophoresis. For prenatal diagnosis, gels were first hybridised to the β^A probe and then, after washing, to the β^H probe. In cases at risk for a genetic compound of two different mutations, gels were first hybridised to an oligonucleotide probe complementary to one mutation and then, after washing, to an oligonucleotide probe complementary to the second mutation.

Those cases not defined by oligonucleotide hybridisation were tested either with *Rsa*I to detect the C–G substitution at position 745 of IVS–2 of the β globin gene (β^+ IVS–2 nt 745),⁴ which creates a new site for this enzyme, or with *Mst*II to identify the mutation at the β^6 position (frameshift 6) of the β globin gene (β^6) which removes an *Mst*II site.²¹

Results

A representative autoradiograph of the prenatal diagnosis in one case at risk for the genetic compound of two different β thalassaemia mutations is shown in the figure. The overall results are summarised in table 1. The mutation was defined in both parents in 47 cases and in one parent only in 12 cases. The most frequent mutations in decreasing order of frequency were β^{39} , β^+ IVS–1 nt 110, β^0 IVS–1 nt 1, β^+ IVS–1 nt 6 (table 2). In two couples we were unable to define the mutation using this approach.

Prenatal diagnosis was accomplished successfully in all cases in which the β thalassaemia mutation was defined in both parents, and by exclusion in one case of those (four cases) in which the mutation was

FIGURE Prenatal diagnosis of β thalassaemia. Autoradiograph of gels hybridised to oligonucleotide probes complementary to the β^+ IVS–1 nt 110 and β^{39} mutations. Lane 1: normal control. Lane 2: father heterozygous for the β^+ IVS–1 nt 110 mutation. Lane 3: mother heterozygous for the β^{39} mutation. Lane 4: affected fetus, genetic compound for both mutations. Lane 5: subject homozygous for β^+ IVS–1 nt 110 mutation (top) and subject homozygous for β^{39} mutation (bottom).

TABLE 1 Definition of the β thalassaemia mutation in prospective parents of Italian descent

	No	%
Total no examined	61	
Both parents	47	77
One parent	12	20
None	2	3

TABLE 2 β thalassaemia mutations in prospective parents of Italian descent.

β globin genotype	No	%
β^{39}	47	38.5
β^+ IVS-1 nt 110	32	26.2
β^+ IVS-1 nt 6	9	7.4
β^+ IVS-1 nt 1	8	6.6
β^+ IVS-2 nt 745	6	4.9
β^6	3	2.5
$\delta\beta$ deletion	1	0.8
Hb Lepore	1	0.8
Unknown	15	12.3

TABLE 3 Prenatal diagnosis by the direct detection of β thalassaemia mutation

	No investigated	Successful test	
		No	%
Mutation defined in both parents	27	27	100
Mutation defined in one parent	4	1	25

identified in only one parent (table 3). In the remaining cases prenatal diagnosis was carried out by globin chain synthesis analysis on fetal blood.

Discussion

This study shows that use of a limited number of oligonucleotide probes and restriction enzymes allows definition of the β thalassaemia mutation in the vast majority (70%) of prospective parents of Italian descent. This has allowed us successfully to accomplish prenatal diagnosis either by amniocyte or chorionic villus DNA analysis. Without this approach, the only method available for prenatal diagnosis in these couples would have been fetal blood analysis, which is associated with a high risk (5%) of fetal mortality.²² Prenatal diagnosis was also carried out successfully by exclusion in one out of four cases (25%) in which the mutation had been defined in only one parent.

Since the distribution and prevalence of these mutations is different in the various high risk areas of Italy,¹⁴ we relied on the origin of the prospective parents to select the sequence of the oligonucleotide probes or restriction enzymes to be used for screening.

Definition of the relative prevalence of less frequent β thalassaemia alleles in Italians may increase the proportion of childless couples whose pregnancies can be monitored by DNA analysis.

The approach followed in this study is also relevant to other Mediterranean populations, in which the β^{39} , β^+ IVS-1 nt 110, β^+ IVS-1 nt 1, and

β^+ IVS-1 nt 6 are also the most prevalent β thalassaemia mutations, although with different distributions in each population.⁴⁻¹⁵

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