β thalassaemia mutations in Turkish Cypriots

A SOZUOZ*, A BERKALP*, A FIGUS†, A LOI‡, M PIRASTU‡, AND A CAO§

From *the Turkish Cypriot Thalassaemia Control Program, Nicosia, Cyprus; †Istituto di Medicina Interna, Clinica Medica 1, and §Istituto di Clinica e Biologia dell'Età Evolutiva, Università degli Studi di Cagliari; and ‡Istituto di Ricerca sulle Talassemie e Anemie Mediterranee Consiglio Nazionale delle Ricerche, Cagliari, Sardinia, Italy.

SUMMARY Using oligonucleotide hybridisation or restriction endonuclease analysis, we have characterised the molecular defect in 94 patients with thalassaemia major and four with thalassaemia intermedia of Turkish Cypriot descent. We found that four mutations, namely β^+ IVS-1 nt 110, β^0 IVS-1 nt 1, β^+ IVS-1 nt 6, and β^+ IVS-2 nt 745 were prevalent, accounting for 69·9%, 11·7%, 8·7%, and 5·6% respectively of the β thalassaemia chromosomes. This information may help in the organisation of a large scale prevention programme based on fetal diagnosis of β thalassaemia by DNA analysis in the Turkish population.

In the last few years the molecular basis of β thalassaemia has been defined and more than 40 different molecular defects have been characterised so far.[1-5] In spite of this heterogeneity, in all populations studied a limited number of molecular defects is usually prevalent.[2-4 6-12] Of the population of Cyprus, 18% are heterozygous for a major haemoglobinopathy[13]: 16% carry β thalassaemia trait, 1% α thalassaemia trait, and 1% Hb S trait. The corresponding birth rate of homozygotes for β thalassaemia is 1 in 144.

Prevention programmes based on community education, premarital heterozygote screening, counselling, and prenatal diagnosis have proved highly acceptable to both Turkish and Greek Cypriots.[14] As a result, the birth rate of thalassaemia major has fallen to less than 5% of its expected value throughout the island.[15] Heterozygote screening is readily available and second trimester prenatal diagnosis using conventional protein methods is well established. However, this depends on fetal blood sampling at 18 to 19 weeks of pregnancy, with attendant disadvantages, and it is desirable to move to first trimester prenatal diagnosis using CVS and fetal DNA analysis, in order to offer couples earlier relief and simpler first trimester abortion when indicated.

A critical prerequisite for prenatal diagnosis by DNA analysis in prospective parents from a given population is the knowledge of the prevalence and distribution of β thalassaemia mutations. In the present study, we have defined β thalassaemia mutations by oligonucleotide hybridisation and restriction endonuclease analysis in 98 Turkish Cypriots with thalassaemia major or intermedia.

Patients and methods

PATIENTS

Peripheral blood samples were collected from 94 patients with thalassaemia major and four with thalassaemia intermedia. All these patients are of Turkish Cypriot extraction and are being followed at present in the Paediatric Department of the Nicosia Turkish Hospital.

METHODS

Oligonucleotide analysis
In order to define the β thalassaemia mutations, oligonucleotide hybridisation with four oligonucleotide probes complementary to the most common mutations in Mediterranean people[1 16] (β^+ IVS-1 nt 110, β^0 IVS-1 nt 1, β^+ IVS-1 nt 6, and $\beta^0$39) was carried out, as previously described,[17] in each patient. Briefly, 10 µg genomic DNA were digested with the restriction endonuclease *Bam*HI according to the recommendations of the manufacturer (Amersham) and DNA fragments were separated by agarose gel electrophoresis. Each mutation was analysed with two oligonucleotide (19mers) probes, one complementary to the β globin gene sequence around the mutation (β^{th} probe) and one homolo-

gous to the normal β globin gene sequence at the same position (β^A probe). They differ from one another in a single nucleotide placed in the middle of the sequence. All β thalassaemia mutations investigated reside in the 1·8 kb *Bam*HI fragment which contains the 5' part of the β globin gene. The sequences of the oligonucleotides used are:

β$^+$ IVS-1 nt 110	βth probe 5' CTCTGCCTATTAGTCTATT 3'
	β^A probe 5' AATAGACCAATAGGCAGAG 3'
β$^°$ IVS-1 nt 1	βth probe 5' CCTGGGCAGATTGGTATCA 3'
	β^A probe 5' TGATACCAACCTGCCCAGG 3'
β$^°$ 39	βth probe 5' AGAACCTCTAGGTCCAAGG 3'
	β^A probe 5' CCTTGGACCCAGAGGTTCT 3'
β$^+$ IVS-1 nt 6	βth probe 5' GCAGGTTGGCATCAAGGTT 3'
	β^A probe 5' AACCTTGATACCAACCTGC 3'

Restriction endonuclease analysis

Restriction endonuclease analysis was carried out according to the method of Goossens and Kan.[18] Haplotype analysis at the β globin gene cluster was carried out in all β thalassaemia chromosomes where we were unable to identify the mutation by oligonucleotide hybridisation. For the haplotype determination, the following polymorphic restriction enzyme sites were studied: *Hinc*II 3' to the ε globin gene,[19] *Hind*III within the Gγ and Aγ globin genes,[20] *Hinc*II within and 5' to the Ψβ globin gene,[19] *Ava*II within the second intervening sequence of the β globin gene,[21] and *Bam*HI 3' to the β globin gene.[22]

Chromosomes with haplotype VII (according to the nomenclature of Orkin *et al*[16]) were tested with the restriction enzyme *Rsa*I in order to detect the β$^+$ IVS-2 nt 745 mutation, which creates a new site for this enzyme and is frequently linked to haplotype VII.[16]

Results

We were able to define β thalassaemia mutations in 191 out of 196 chromosomes investigated (table 1). The most common defect, accounting for 69·9% of β thalassaemia chromosomes, was β$^+$ IVS-1 nt 110 mutation, followed by β$^°$ IVS-1 nt 1, β$^+$ IVS-1 nt 6, β$^+$ IVS-2 nt 745, and β$^°$39, which were found in 11·7%, 8·7%, 5·6%, and 1·5% respectively of β thalassaemia chromosomes.

TABLE 1 *β thalassaemia mutations in Turkish Cypriots.*

Mutations	No of positive chromosomes	%
β$^+$ IVS-1 nt 110	137	69·9
β$^°$ IVS-1 nt 1	23	11·7
β$^+$ IVS-1 nt 6	17	8·7
β$^°$39	3	1·5
β$^+$ IVS-2 nt 745	11	5·6
Undefined	5	2·6
Total No of chromosomes examined	196	100

TABLE 2 *β globin genotype of Turkish Cypriot β thalassaemia patients.*

Mutations	Patients		%
β$^+$ IVS-1 nt 110/β$^+$ IVS-1 nt 110	50	(2)*	51·02
β$^+$ IVS-1 nt 110/β$^+$ IVS-1 nt 6	13		13·60
β$^+$ IVS-1 nt 110/β$^°$ IVS-1 nt 1	14		14·30
β$^°$ IVS-1 nt 110/β$^°$39	1		1·02
β$^°$ IVS-1 nt 1/β$^°$ IVS-1 nt 1	3		3·06
β$^+$ IVS-1 nt 6/β$^+$ IVS-1 nt 6	1	(1)	1·02
β$^+$ IVS-1 nt 110/β$^+$ IVS-2 nt 745	7		7·14
β$^+$ IVS-2 nt 745/β$^+$ IVS-2 nt 745	1		1·02
β$^°$ IVS-1 nt 1/β$^+$ IVS-1 nt 6	2	(1)	2·04
β$^°$39/β$^+$ IVS-2 nt 745	1		1·02
β$^°$ IVS-1 nt 1/β$^+$ IVS-2 nt 745	1		1·02
β$^°$39/undefined	1		1·02
β$^+$ IVS-1 nt 110/undefined	2		2·04
Undefined/undefined	1		1·02
Total No of patients	98		100

*The number of thalassaemia intermedia patients with the defined genotype is indicated in brackets.

Fifty of the patients investigated (51%) were homozygous for β$^+$ IVS-1 nt 110 mutation, three for β$^°$ IVS-1 nt 1 (3%), one for β$^+$ IVS-1 nt 6 (1%), and one for the β$^+$ IVS-2 nt 745 (1%) mutation (table 2). The remaining were compound heterozygotes for two different mutations. The most frequent combinations were β$^+$ IVS-1 nt 110/β$^+$ IVS-1 nt 6 (13·6%) and β$^+$ IVS-1 nt 110/β$^+$ IVS-1 nt 1 (14·3%).

Of the four patients with the clinical phenotype of thalassaemia intermedia, two were homozygous for β$^+$ IVS-1 nt 110, one was homozygous for β$^+$ IVS-1 nt 6, and one was a genetic compound for β$^°$ IVS-1 nt 1/β$^+$ IVS-1 nt 6.

Of the 16 chromosomes in which oligonucleotide analysis failed to identify the mutation, 11 were associated with haplotype VII and by restriction analysis with *Rsa*I all of them were found to carry β$^+$ IVS-2 nt 745 mutation. The remaining five undefined β thalassaemic genes were associated with the following haplotypes: ++−−++ (3); ++−++++ (1); −−−−−+ (1).

Discussion

This study shows that in the Turkish Cypriot population, a limited number of β thalassaemia mutations are prevalent. A similar trend has been detected in all populations at risk investigated so far. In the Turkish Cypriots, a single mutation, the β$^+$ IVS-1 nt 110, accounts for 70% of the β thalassaemia chromosomes. In the remaining chromosomes in which the mutation was defined, we detected the β$^°$ IVS-1 nt 1 (11·7%), the β$^+$ IVS-1 nt 6 (8·7%), the β$^+$ IVS-2 nt 745 (5·6%), and the β$^°$39 (1·5%) mutations.

These defects may be directly detected by oligonucleotide hybridisation or, in the case of β$^+$ IVS-1 nt 745 mutation, by restriction endonuclease analysis. A

limited number of oligonucleotide probes, complementary to the most prevalent mutations in this population, allows prenatal diagnosis in 95% of the couples at risk for thalassaemia major.

As regards the correlation between specific mutation and clinical phenotype, we confirmed that the homozygous state for β^+ IVS-1 nt 6 mutation produced the clinical phenotype of thalassaemia intermedia. β^+ IVS-1 nt 6 mutation, however, in combination with the β^+ IVS-1 nt 110 or β° IVS-1 nt 1 mutation, results in the clinical phenotype of thalassaemia major or intermedia. We have no explanation for the intermedia phenotype detected in two homozygotes for β^+ IVS-1 nt 110, as the presence of other ameliorating factors such as α thalassaemia or HPFH determinants[23] associated with increased Hb F in the heterozygous state was excluded.

The results obtained in this study will help in the organisation of a large scale prevention programme based on fetal diagnosis of thalassaemia by DNA analysis in Turkish Cypriot couples.

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Correspondence and requests for reprints to Professor Antonio Cao, Istituto di Clinica e Biologia del'Età Evolutiva, Università degli Studi, c/o Ospedale Regionale Microcitemie, Via Jenner s/n, I-09100 Cagliari, Sardinia, Italy.