Red Cell Disorders

The molecular basis of β-thalassemia in Argentina. Influence of the pattern of immigration from the Mediterranean Basin

In order to determine the molecular heterogeneity of the β-thalassemia gene and to analyze the influence of immigration from the Mediterranean Basin, a total of 254 families (475 subjects) from Argentinean β-thalassemia patients were investigated using molecular biology techniques. This allowed us to provide a simplified diagnosis and genetic counselling of this disorder in Argentina.

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β-thalassemia is a common hereditary disorder characterized by an inability to synthesize an adequate amount of β-globin chains. To date, almost 200 β-thalassemic alleles have been characterized, for the most part resulting from point mutations.[1] The spectrum of mutations differs among ethnic groups in each population and includes a few common mutations and a variable number of rare ones.[2]

β-thalassemia is a major genetic problem in many Mediterranean countries. Historically, the Spanish colonization was mainly responsible for the introduction of β-thalassemic genes in Argentina. Subsequent immigration waves from the Mediterranean Basin, particularly from Italy and Spain, during the nineteenth and twentieth centuries, also contributed greatly. The number of immigrants quickly equalled the number of natives and a mixed population resulted. Previous hematologic studies showed a 0.8% incidence of thalassemic carriers among 4000 blood donors living in the Great Buenos Aires area.[3]

We studied 254 families with β-thalassemia from different areas of Argentina. The total number of subjects investigated was 475. Of these, 350 were carriers of β-thalassemia (228 unrelated chromosomes), 24 had thalassemia major and 2 had thalassemia intermedia; the remaining 99 samples (from relatives) showed normal hematologic data. The ethnic background of the patients studied was: 65.1% Italians, 25.4% Spaniards, 6.0% from Arabian countries, 2.5% from Greece and 1.0% from Portugal.

Direct point mutations were identified by polymerase chain reaction (PCR) with allele-specific oligonucleotide (ASO) hybridization or restriction fragment length polymorphism (RFLP). Patients with unidentified DNA were submitted to single-stranded conformation polymorphism (SSCP) screening, and the samples that displayed an aberrant banding pattern were then subjected to DNA-sequencing (Table 1).[4]

Molecular characterization of β-thalassemia mutations was achieved in 262 out of 280 chromosomes (93.5 %) by using PCR-RFLP or PCR-ASO techniques (Table 1). The most frequently encountered mutations were codon 39(C-T), IVS-I-110(G-A), IVS-I-1(G-A) and IVS-I-6(T-C) which were identified in 87.6% of the alleles (Table 2).

Fourteen β-thalassemic carriers and 3 thalassemia major patients (2 patients with one unidentified mutation, and one patient with both unidentified mutations), underwent SSCP-screening. Five samples (4 from β-thalassemia carriers, 1 from a thalassemia major patient) displayed an abnormal electrophoretic pattern. All these patients and their relatives were submitted to genomic DNA-sequencing. Codon 30(G-C) was present in two β-thalassemia carriers of Iranian and Syrian-Lebanese origin. Codon 44(-C) was found in another β-thalassemia carrier of Syrian-Lebanese origin and codon 8-9(+G) was present in a β-thalassemia carrier of Syrian origin. IVS-I-5(G-C) was found by SSCP sequencing in the thalassemia-major patient in association with an IVS-I-6(T-C). This mutation was also found by direct sequencing in another patient of Italian origin with thalassemia-major. In addition to the mutations found, we incidentally detected several common polymorphisms by SSCP. After sequencing, the IVS-II-666(T-C) and different associations

Table 1. Strategy for direct characterization of β-thalassemic point mutations.

Mutation	*PCR product used*	*Strategy*
-87 (C–G)	A	PCR-RFLP[a]
Codon 6 (–A)	A or C	PCR-RFLP[b]
Codon 39 (C–T)	A or C	ASO
IVS-I-1 (G–A)	A or C	ASO
IVS-I-6 (T–C)	A or C	ASO
IVS-I-110 (G–A)	A or C	ASO
IVS-II-1 (G–A)	A	ASO
IVS-II-745 (C–G)	B	ASO or PCR-RFLP[c]
Unknown	B - C and D	SSCP – SEQ.

A The PCR product was 720 bp long, Forward oligonucleotide sequence (F): P1: 5'- TAAGCCAGTGCCAGAAGAGCC - 3' Reverse oligonucleotide sequence (R): P2: 5'- TCCTATGACATGAACTTAAC - 3'. B PCR product (664 bp); F: P3: 5'- CTAATCTCTTTCTTTCAGGG - 3'; R: P4: 5'- TAGTGTATTTTCCCAAGGTT - 3'. C PCR product (536 bp), F: P5: 5'- GGTTGGCCAATCTACTCCCAGG - 3'; R: P6: 5'- GCTCACTCAGTGTGGCAAAG - 3'. D PCR product (227 bp); F: L1: 5'- TCATGGCAAGAAAGTGCTCG - 3'; R: L4: 5'- CCCATTCTAAACTGTA CC - 3'.
PCR-RFLP: amplification + endonuclease restriction; ASO: allele-specific oligonucleotide dot hybridization; SSCP-SEQ.: SSCP screening and sequencing. a: Avr II restriction; b: Dde I restriction; c: Rsa I restriction.

Table 2. Frequency distribution of 12 β-thalassemia alleles in the Argentinean population and Mediterranean regions from where major immigratory waves originated.

Mutation	*Type*	*This study*	*Italy[#] (6-7)*	*Sardinia (9)*	*Sicily (6-8)*	*Spain (10)*
-87 (C–G)	β^+	0.7	1.7	0.2	2.0	–
Codon 6 (–A)	β^0	2.1	2.0	2.1	1.6	0.3
Codon 8-9 (+G)	β^0	0.4	–	–	–	9.4
Codon 30 (G–C)	β^0	0.7	–	0.2	0.5	–
IVS-I-1 (G–A)	β^0	10.7	7.9	0.1	8.2	29.5
IVS-I-5 (G–C)	β^+	0.7	0.1	-	0.5	1.0
IVS-I-6 (T–C)	β^+	7.9	14.4	0.1	16.0	8.4
IVS-I-110 (G–A)	β^+	23.2	21.0	0.5	23.6	8.1
Codon 39 (C–T)	β^0	45.7	38.8	95.6	35.5	31.2
Codon 44 (–C)	β^0	0.4	0.4	–	–	–
IVS-II-1 (G–A)	β^0	2.5	3.9	0.1	1.6	0.3
IVS-II-745 (C–G)	β^+	0.7	4.6	0.4	5.8	1.7
Unknown		4.3	2.9	–	2.1	4.5
Others		–	2.3	0.7	2.6	5.6
Total		100.0	100.0	100.0	100.0	100.0
(N. of chrom.)		(280)	(1641)	(3000)	(1299)	(308)

[#]The average of frequencies found in Calabria, Basilicata, Apulia, Campania, Latium, the Po Delta and Genoa are considered in this column.

of IVS-II-16(C-G), IVS-II-74(G-T), and IVS-II-81(C-T), codon 2(C-T) and the 5´UTR+20(C-T) changes were found.[5]

Direct mutation detection techniques in association with SSCP-mutation screening and sequencing allowed characterization of the mutation in almost all cases (95.7%).

As many as 9 out of the 24 thalassemia-major patients studied were homozygous (38%), 6 of them for codon 39(C-T), and 3 of them for the IVS-I-110(G-A) mutation; the other 15 subjects were compound heterozygotes, with combinations mostly involving codon 39, and IVS-I-110.

Homozygosity for the mild IVS-I-6(T-C) allele was encountered in 2 unrelated patients of Italian origin with thalassemia-intermedia.

Argentina has been the site of important immigratory currents, particularly from Italy and Spain, but also, to a smaller extent, from Arabian countries. When the ethnic origin of the patients studied was established, this allowed us to compare our results with those in the countries of origin.[2] The values of the most common alleles are in between those seen in the other populations. The four most frequent mutations present in our study, codon 39(C-T), IVS-I-110(G-A), IVS-I-1(G-A) and IVS-I-6(T-C) (bold typed in Table 2), accounting for 87.6% of all mutations, correspond to about 77 to 96% of the anomalies in the populations of origin. The prevalence of the fifth most common mutation varies among the various countries or regions.

Four uncommon mutations which had not been previously reported in Argentina were found in 6 β-thalassemia families, mostly of Arabian origin. Codon 30(G-C) is a rare β^0-thalassemic mutation in Mediterranean populations[2] and has been described among the Gond tribal groups of central India. Codon 44(-C) is the most prevalent allele among Kurdish Jews. IVS-I-5(G-C) was previously found in Chinese, Asian Indian and Mediterranean populations.[1] Codon 8-9(+G) is a common allele in Asian-Indians but is rare in Mediterranean regions, although a relatively high frequency (9.4%) was found in Spain.[10]

The data presented here update the frequency of β-thalassemic mutations in our country; this will be useful for genetic counselling, carrier-screening and the establishment of a comprehensive antenatal diagnosis program. Until inexpensive and effective treatment becomes available, prevention will continue to have the greatest impact on the burden of hemoglobinopathies worldwide.

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