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ORIGINAL ARTICLE

MOLECULAR SPECTRUM OF β-THALASSEMIA MUTATIONS IN THE ADMIXED VENEZUELAN POPULATION, AND THEIR LINKAGE TO β-GLOBIN GENE HAPLOTYPES

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□ *In order to establish the spectrum of β-thalassemia (β-thal) mutations in the Venezuelan population for the first time, 127 unrelated subjects either with a suspicion of β-thal trait or with a clinically recognized β-thal syndrome of different degrees of severity, were studied. DNA from these subjects was analyzed by a polymerase chain reaction (PCR)-based reverse dot-blot method or amplification refractory mutation system (ARMS). Prototype β-globin gene sequencing of relevant DNA was performed to confirm the mutations. Fifteen different mutations were identified accounting for 92.0% of the mutant alleles explored, revealing a significant genetic heterogeneity at the β-globin gene locus in this population. The most frequent mutations were codon 39 (C>T) 34.1%, IVS-I-1 (G>A) 11.1%, IVS-I-6 (T > C) 6.6%, IVS-I-110 (G >A) 6.6%, IVS-II-849 (A >G) 6.6%, −88 (C >T) 6.0%, −29 (A>G) 5.2%, followed by the less common IVS-I-5 (G>A) 3.7%, the 1,393 bp deletion 3.0%, IVS-II-1 (G>A) 3.0%, −86 (C>G) 2.2%, IVS-II-1 (G>T) 1.5%, codons 41/42 (–TCTT) 1.5%, IVS-II-745 (C >G) 0.7% and deletional δβ-thal 0.7%. Overall, these data demonstrate that the major sources of β-thal alleles in Venezuela, as expected, are of Mediterranean and African origins. This is the first large study defining the molecular spectrum of β-thal in the highly admixed population of Venezuela and lays the foundation for genetic counseling as well as implementing comprehensive*

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clinical care programs. Diversity of haplotypes associated with some of the β-thal mutations can be explained by in situ *recombination events in Venezuela.*

Keywords β-Thalassemia (β-thal) mutations, Hb S-β-thalassemia

INTRODUCTION

β-Thalassemia (β-thal) is a highly heterogeneous autosomal recessive disorder (1). In Venezuela, β-thal trait had been found in native families established for several generations, in subjects of recent migration with Portuguese, Italian, French and Spanish ancestry and also in the Mestizo population resulting from the admixture of Amerindians, Africans and Caucasians (2–4). The Venezuelan Amerindians do not have abnormal hemoglobin (Hb) variants *per se*, and the rare cases encountered are believed to be due to gene flow from the above mentioned admixture (5–7). In addition to β-thal alleles, the presence of Hb S [β6(A3)Glu→Val, G*A*G>G*T*G], Hb C [β6(A3)Glu→Lys, *G*AG>*A*AG] and hereditary spherocytosis, has also been reported in this population (8–10). Due to the copresence of both the sickle cell gene and β-thal alleles in the same population it is not infrequent to encounter a significant number of sickle cell β-thal cases. In this study we have focused our attention to determine the molecular spectrum of β-thal alleles in a hospital-based population, which is a prerequisite to implement appropriate prevention and control programs.

Thus far, more than 200 β-thal alleles have been identified worldwide, and each population has a unique spectrum of mutations consisting of a few very common ones and a variable number of rare ones (11-14). More than 95.0% of the genetic defects responsible for β-thal are point mutations negatively affecting the β-globin gene expression, while β-thalassemic alleles due to deletions of variable size are rare (1).

In the Mediterranean population, the most frequent mutations are the codon 39 (C>T) nonsense mutation and mutations affecting the splicing process, namely, IVS-I-110 (G>A), IVS-I-6 (T>C) and IVS-I-1 (G>A) (15–19). In the African population, the most common β-thal alleles are the A>G substitution at position −29 in the TATA box and the C>T substitution at −88 (20,21). In the Asian population, the most frequent alleles are the codons 41/42 (–TCTT), codons 8/9 (+G), 619 bp deletion, codon 17 (A>T), −28 (A>G), IVS-II-654 (C>T), codons 71/72 (+A), IVS-I-5 (G>C) and IVS-I-1 (G>T) (22–25).

Given this information as well as the history of the admixture in our population, we anticipated that the spectrum of β-thal mutations in our population must be wide. Here we demonstrate that of the 15 different β-thal alleles present, seven were more than 5.0% and six were more than 1.0%; the mutational profile corresponds to the input of various influx populations. Nevertheless, a significant proportion (7.5%) of mutations yet remain to be defined.

MATERIALS AND METHODS

Blood samples were collected from 127 unrelated individuals, all self-declared as Mestizos (excepting two Chineses and one Lebanese) and suspected to have either β-thal trait or a clinically recognized thalassemia syndrome, and processed at the Laboratorio de Investigación de Hemoglobinas Anormales, Universidad Central de Venezuela, Caracas, Venezuela. The trait status for β-thal was based on combined microcytic (MCV<76 fL) and hypochromic (MCH<24 pg.) features of the red blood cell and a Hb A_2 level of >4.0% and for β-thal intermedia (β-TI) by the presence of anemia, a pathological blood smear and with or without splenomegaly and/or occasional transfusion requirements. All the β-thal major (β-TM) patients had severe anemia, splenomegaly and transfusion dependency from an early age. Consent was obtained from all participants in this study and they represented different subpopulation groups of Venezuela, namely Mestizos, Amerindians, Caucasians and Africans.

Hematological parameters were measured using automated cell counters (Coulter Counter model T-660; Coulter Corporation, Hialeah, FL, USA). Quantitation of Hb A, Hb A_2 and Hb F was performed by ion exchange high performance liquid chromatography (HPLC) (VARIANT™, Bio-Rad Laboratories, Hercules, CA, USA) using the β-Thalassemia Short Program (26). Family studies were performed wherever possible.

Genomic DNA was isolated from white blood cells by the salting-out procedure (27). Analysis of the β-globin gene mutations was done by a polymerase chain reaction (PCR)-based reverse dot-blot technique, employing 30 different oligonucleotide probes (28) and when needed, by amplification refractory mutation system (ARMS) (29). The confirmation of the mutation status was carried out by the prototype nucleotide sequencing, using the BigDye™ Terminator Cycler Sequencing Kit and the ABI PRISM™ 310 Genetic Analyzer (PE Applied Biosystems, Foster City, CA, USA) (30). The 1,393 bp deletional allele was detected by an established PCR amplification procedure (31).

The β-globin gene cluster haplotype was characterized by a PCR-based restriction enzyme analysis (PCR-RFLP) (restriction fragment length polymorphism) (32,33). Seven restriction sites were analyzed including the *Hind*II (5′ of ε-globin gene), *Hind*III (intron 2 of the $^{G}\gamma$ gene), *Hind*III ($^{A}\gamma$-globin gene), *Hind*II (5′ of the ψβ-globin gene), *Hind*II (3′ of the ψβ-globin gene), *Ava*II (intron 2 of β-globin gene) and *Hinf*I (3′ of the β-globin gene). The sickle cell β-thal patients were subclassified into Hb S-β0-thal with absent production of Hb A and Hb S-β^{+}-thal types with variable proportion of Hb A (1).

RESULTS

We analyzed the molecular spectrum of β-thal in 127 unrelated Venezuelan subjects, essentially consisting of the admixed Mestizos. Given

the history of this population subgroup, we targetted our screening strategy to the most common mutations described to be prevalent in the African and Mediterranean populations. Thus, we were able to identify the β-thal mutations in 92.0% of the total number of alleles studied.

We detected 15 different mutations (Table 1). Eighty-three alleles carried a β0-thal mutation and 42 carried β^+-thal mutation. The codon 39 nonsense mutations is the most common defect, followed by IVS-I-1, IVS-I-6, IVS-I-110 and IVS-II-849 alleles. The less common mutations found were −88, −29, IVS-I-5, 1,393 bp deletion, IVS-II-1, −86, IVS-II-1, codons 41/42, IVS-II-745 and deletional δβ-thal alleles. These mutation represented 92.5% of the total thalassemic alleles with the remaining 7.5% yet to be defined.

Of the 127 subjects studied, 91 were β-thal trait subjects, three with β-TI, five with β-TM and 25 with compound heterozygosities for Hb S-β-thal alleles. In addition, three cases were found to be compound heterozygotes for Hb C-β-thal.

Of the three β-TI patients, two were homozygotes for −88 and one for the IVS-I-6 mutations. Of the five β-TM patients, two were homozygotes for codon 39, one patient each for IVS-I-1 and for IVS-I-6 mutations; the remaining patient was a compound heterozygote for IVS-II-849 and δβ-thal alleles.

In the Hb S-β^+-thal group ($n = 10$), we found the mild A>G substitution at position −29 in the TATA box, along with the −86, −88, IVS-II-745, IVS-I-6, IVS-I-110 and IVS-I-5 mutations. Five different molecular defects were characterized in the Hb S-β0-thal group ($n = 15$), the codon 39 mutation being the most frequent along with the 1.393 bp deletion, IVS-II-849, IVS-I-1 and IVS-II-1

TABLE 1 Spectrum and Frequency of β-Thalassemia Mutations in Venezuela

Mutations	Type	Number of Alleles	Frequency (%)	Ethnic Origin
Codon 39 (T>C)	β○	46	34.1	Mediterranean
IVS-I-1 (G>A)	β○	16	11.1	Mediterranean
IVS-I-6 (T>C)	β^+	9	6.6	Mediterranean
IVS-I-110 (G>A)	β^+	9	6.6	Mediterranean
IVS-II-849 (A>G)	β○	9	6.6	African
−88 (C>T)	β^+	8	6.0	African
−29 (A>G)	β^+	7	5.2	African
IVS-I-5 (G>A)	β^+	5	3.7	Mediterranean
IVS-II-1 (G>A)	β○	4	3.0	Mediterranean
1,393 bp deletion	β○	4	3.0	African
−86 (C>G)	β^+	3	2.2	Lebanese
IVS-II-1 (G>T)	β○	2	1.5	Surinamese
Codons 41/42 (–TCTT)	β○	2	1.5	Chinese
IVS-II-745 (C>G)	β^+	1	0.7	Mediterranean
δβ-Thalassemia	β○	1	0.7	Mediterranean
Unidentified		10	7.5	
Total		135	100.0	

TABLE 2 Haplotypes Linked With β-Thalassemia Mutations in Venezuela

Mutation	Number of Alleles	*Hind*II (5′ to ε)	*Hind*III ($^{G}\gamma$)	*Hind*III ($^{A}\gamma$)	*Hinc*II (ψβ)	*Hinc*II (3′ to ψβ)	*Ava*II (β)	*Hinf*I (3′ to β)	Haplotype[a]
Codon 39 (T>C)	11	[+]	[−]	[−]	[−]	[−]	[+]	[+]	I
	8	[−]	[+]	[+]	[−]	[+]	[+]	[+]	
IVS-I-6 (T>C)	5	[−]	[+]	[+]	[−]	[−]	[−]	[+]	VI
	2	[+]	[−]	[−]	[−]	[+]	[−]	[+]	
	1	[−]	[+]	[−]	[−]	[+]	[−]	[+]	
IVS-I-110 (G>A)	7	[+]	[−]	[−]	[−]	[−]	[+]	[+]	I
1.393 bp deletion	4	[+]	[−]	[−]	[−]	[−]	[+]	[+]	I
−88 (C>T)	5	[−]	[−]	[−]	[−]	[+]	[+]	[+]	–
	1	[+]	[−]	[−]	[−]	[+]	[+]	[+]	
IVS-I-1 (G>A)	7	[+]	[−]	[−]	[−]	[−]	[+]	[−]	V
IVS-I-5 (G>A)	3	[+]	[−]	[−]	[−]	[−]	[−]	[+]	VII
IVS-II-1 (G>A)	3	[−]	[+]	[−]	[+]	[+]	[+]	[−]	III
−29 (A>G)	3	[−]	[−]	[−]	[+]	[+]	[+]	[+]	IX
	1	[−]	[+]	[−]	[+]	[+]	[+]	[+]	
	1	[−]	[−]	[−]	[−]	[−]	[+]	[+]	
	1	[−]	[−]	[−]	[−]	[+]	[+]	[+]	
IVS-II-849 (A>G)	2	[−]	[−]	[+]	[−]	[+]	[−]	[+]	–
	1	[−]	[+]	[+]	[−]	[+]	[−]	[+]	
IVS-II-745 (C>G)	1	[+]	[−]	[−]	[−]	[−]	[−]	[+]	VII
−86 (C>G)	2	[+]	[−]	[−]	[−]	[−]	[+]	[+]	I
IVS-II-1 (G>T)	1	[−]	[−]	[−]	[−]	[+]	[+]	[+]	–

[a]Haplotype classification according to Orkin *et al.* (33) and Old (29).

alleles. The β-thal alleles were found to be associated with one or more haplotypes (Table 2).

DISCUSSION

This is the first study to determine the profile of β-thal mutations in the Venezuelan admixed population, which is a very heterogeneous one due to centuries of inter ethnic admixture of peoples from three different continents, namely, European colonizers, (mainly represented by the Spaniards), African slaves and Amerindians (34). In the last century, significant immigration of Italians, Portuguese and Spaniards has taken place in the period extending from 1940 to 1975. Much more recently, increasing numbers of immigrants arrived from neighboring countries such as Colombia, Ecuador, Dominican Republic and Haiti. Although some North European immigration from The Netherlands, Germany, Poland and France occurred, their contribution to the genetic makeup remained minimal (4).

The present study reveals that the Venezuelan population carried at least 15 different β-thal mutations, all of which have been reported previously in the African and Mediterranean areas (35,36). The finding that the codon 39 mutation is the most frequent one, followed by other African mutations, is

in line with the history of the population of Venezuela. Indeed, the present day Venezuelan population is the result of admixtures between different groups at different time periods; the colonizing Spaniards mixed with the indigenous populations as well as with African slaves during three centuries. Later, immigration from Spain, Italy, Portugal contributed further to the admixture of the present-day Venezuelans.

We compared the frequency of β-thal mutations detected in this study with other data reported for Central and South American populations such as Brazilians (37–40), Cubans (41), Guadeloupians (42), Argentineans (43) and Mexicans (44) (Table 3). The very high frequency of the codon 39 mutation observed in Venezuelans is similar to that reported for countries with a large Caucasian population. The IVS-I-1, the second most frequent mutation in our subjects, is also frequent among the Spaniards (35) and Rio Grande do Norte in Brazil (40) and in Mexico (44). The presence of the IVS-I-6 mutation appears to be a contribution from the Portuguese to the genetic makeup of the population of Venezuela and Rio Grande do Norte in Brazil (40).

The African mutations found in this study were the −29 in the TATA box, the −88, the IVS-II-849 and the 1,393 bp deletion. The −86 mutation, herein described, had also been previously reported in Lebanese patients. Two recent immigrants born in China but living in Venezuela had the codons 41/42 mutation in the heterozygous state.

Indeed, (β^+) IVS-I-6 was originally associated with the β-TI phenotype in the homozygous state and was designated the "Portuguese type." The finding of this mutation in a β-TM Venezuelan patient is intriguing and needs clarification.

Haplotype analysis indicates that the majority of Mediterranean and African mutations are associated with the previously described haplotypes in the original population, reinforcing the gene flow to Venezuela from these regions. Looking at the haplotype association with thalassemia mutation, the following observations are of interest. Five mutations (codon 39, IVS-I-6, −88, −29 and IVS-II-849) are associated with more than one haplotype. Of these, the highest haplotype diversity is associated with the −29 mutation followed by the IVS-I-6 allele. The codon 39, −88 and IVS-II-849 mutations are each associated with two different typical/atypical haplotypes. Despite such haplotype diversity, each mutation is associated with a unique framework sequence. For example, the −29 mutation is associated with four different β-globin gene cluster haplotypes (all carry the framework I pattern) and the IVS-I-6 mutation is associated with three different haplotypes that carry the framework III pattern. This suggests that the observed haplotype divergence is posterior to the mutational event. In principal, the degree of haplotype diversity depends on the antiquity of the mutation as well as diversity of the haplotype encountered during transmission (number of meiotic events). From these findings

TABLE 3 Comparison of the Prevalence of β-Thalassemia Mutations in Central and South American Populations by Country (references in parentheses)

Mutations	Brazil				Cuba (41)	Guadeloupe (42)	Argentina (43)	Argentina (45)	Mexico (44)	Venezuela[a]
	SE (37)	NE (38)	SE 2008 (39)	Rio Grande (40)						
−29 (A>G)	–	–	–	–	13.4	47.3	–	–	–	5.2
−88 (C>T)	–	–	–	–	2.4	3.2	–	–	–	6.0
−86 (C>G)	–	–	–	–	–	–	–	–	–	2.2
Codon 39 (C>T)	64.3	3.5	50.9	–	30.5	–	47.0	45.7	31.4	34.1
IVS-I-1 (G>A)	5.7	15.1	–	48.4	4.9	–	9.4	10.4	14.5	11.1
IVS-I-5 (G>A)	–	–	–	3.2	1.2	10.7	–	–	6.0	3.7
IVS-I-6 (T>C)	7.1	62.8	9.5	41.9	3.7	–	5.9	7.9	–	6.6
IVS-I-110 (G>A)	20.0	8.2	18.1	6.5	8.5	1.1	22.4	23.2	14.5	6.6
IVS-II-1 (G>A)	–	–	12.9	–	8.5	10.7	3.5	2.5	1.2	3.0
IVS-II-745 (C>G)	–	–	–	–	–	–	2.3	0.7	1.2	0.7
IVS-II-849 (A>G)	–	–	–	–	1.2	3.2	–	–	–	6.6
1,393 bp deletion	–	–	–	–	–	–	–	–	–	3.0
IVS-II-1 (G>T)	–	–	–	–	–	–	–	–	–	1.5
Codons 41/42 (–TCTT)	–	–	–	–	–	–	–	–	–	1.5
δβ-Thalassemia	–	–	–	–	–	–	–	–	–	0.7
Others	–	9.5	0.6	–	18.2	23.8	4.7	5.0	31.2	–
Unidentified	2.9	1.1	–	–	7.3	–	4.7	4.3	–	7.5

SE: Southeast; NE: Northeast.
[a]Present study.

we may predict (not prove) that the mutation associated with haplotype heterogeneity might have been introduced earlier into Venezuela than those associated with unique haplotype and/or might have been subjected to larger degrees of admixture. Alternatively, a given mutation might have been introduced into Venezuela from different populations, each associated with different haplotypes In fact, the −29 mutation is quite prevalent in different parts of Africa and the IVS-I-6 allele in Portugal as well as in Lebanon, and may present with different haplotype association in each geographical region.

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