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Spectrum of β Thalassemia Mutations and HbF Levels in the Heterozygous Moroccan Population

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A comprehensive hematological and molecular analysis of 57 β thalassemic heterozygotes, 28 homozygotes, 18 double heterozygotes, 3 compound heterozygotes β thal/β S and one compound heterozygote β thal/Hb Newcastle, in 46 Moroccan families with at least one β thalassemia patient is reported. Six major mutations: $\beta^0$39 (C→T), β^0FsCD8(–AA), β^+IVS1,nt6 (T→C) and β^0IVS1,nt1 (G→A), β^0FsCD6 (–A) and β^+-29 (A→G) cap site account for 75% of the 86 independent β thal chromosomes studied. For the first time, an extensive mutation/haplotype study has been performed on the Moroccan population, and data are consistent with the geographical location of the country and historical links with both the Mediterranean and the Sub-Saharan Africa communities. Despite the heterogeneous spectrum of mutations, good genetic counseling can be offered to the carrier population. This study focuses on the analysis of fetal hemoglobin levels in β thalassemic heterozygotes and its correlation with β globin cluster polymorphic markers in this population. Fetal hemoglobin levels in heterozygotes vary from trace quantities to 17.9% (2.38 g/dl) of total hemoglobin in the adult. No statistically significant correlation was found, either between genders and HbF levels, or between the mutation and the HbF level, with the exception of mutation β^0FSCD6(–A). We have examined the α globin genotype and the β globin genotype of heterozygotes, namely, the extended haplotype, which includes the *Xmn*I site at –158 bp of the Gγ gene and the microsatellite $(AT)_xT_y$ at –540 bp of the β globin gene. In this sample, we confirm the existence of linkage disequilibrium between the C→T variation at –158bp of Gγ globin gene (*Xmn*I$^+$) and Orkin's haplotypes III, IV, or IX (the 5′ subhaplotype class A). At 5′ β globin gene, we observe exclusively the allele $(AT)_7T_7$. In the β thalassemic heterozygotes studied, no correlation of those genetic markers with HbF levels is observed. Am. J. Hematol. 73: 161–168, 2003. © 2003 Wiley-Liss, Inc.

Key words: β thalassemia; mutations; extended haplotype; HbF; HPFH

INTRODUCTION

β Thalassemia, being probably the most common single gene disorder worldwide, has not been fully characterized in the Moroccan population. Morocco is situated in the malaria belt, spanning from the tropics to the Mediterranean basin through Northern Africa, and from the Middle East to South East Asia, thus having an high incidence of this hemoglobinopathy.

β Thalassemia is characterized by a reduced or defective production of the β globin chains, an imbalance in

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the coordinated synthesis of the hemoglobin subunits, triggering the bone marrow to produce immature erythrocytes. This is associated in the patient with variable degrees of anemia, bone marrow hyperplasia, splenomegaly, and other clinical features related to the severity of the anemic state. The synthesis of HbF moderates the α/non-α globin chain imbalance and it attains, in the homozygote, levels of 85%. In the heterozygote, the HbF levels are above 2.5% in the adult, with a dispersion of values up to 14% and are hereditarily transmitted [1,2]. Determinants for this type of hereditary persistence of fetal hemoglobin (HPFH) in normal adults, in β thalassemia and drepanocytosis are multiple and not yet clearly defined [3,4]. Evidence has been provided for the existence of quantitative trait loci influencing F cell number and HbF levels in 6q 22.3-q23.1 [5,6] and at least one other autosomal locus in 8q [7], as well as an F cell production locus in Xp22.2-p22.3 [8,9]. A study with twin pairs of European descent shows that the contribution of β globin cluster elements to the variability of HbF level accounts for about 13% of that variation, and 2% is related to age and sex [6]. Close linkage to the 5′ subhaplotype and to the $(AT)_xT_y$ motif at −540 of the β globin gene in β thalassemia or Hb Lepore heterozygotes [1,10] or in homozygotes [11] of European descent, was also observed.

In the last 20 years, the analysis of β thal chromosomes from various populations led to the characterization of over 325 molecular alterations in the β globin gene, 179 of which cause β thalassemia. At present, the spectrum of β thal mutations and haplotypes in different populations of the Mediterranean basin and near Middle East is clear, except for Morocco.

In Morocco, hemoglobinopathies are a major public health concern due to the high incidence of abnormal hemoglobins (2.61% [12]) and to the high prevalence of malaria in the country. These factors, together with the historical contacts with Sub-Saharan Africa, could explain the upholding of morbid genes in the population, and their amplification derived from endogamy. A screening of the Moroccan population shows that the frequency of β thal (0.95%) opposes the rarity of that of α thal, and that the most frequent abnormal hemoglobins found are HbS (0.59%) and HbC (1.07%) [12,13].

For the first time, an extensive mutation/haplotype study is reported for the Moroccan population, together with the comparison of the phenotypic expression of HbF levels in heterozygotes with other polymorphic markers in 86 independent β thal chromosomes.

MATERIALS AND METHODS

Population Sample

This study included 117 individuals from 46 families with at least one β thal homozygote, 3 genetic compounds β S/β thal, and one genetic compound Hb Newcastle/β thal in a total of 86 β thal independent chromosomes. These families were randomly selected from all over Morocco and referred to us by physicians from the hospitals of Casablanca and Rabat. Homozygotes and compound heterozygotes are transfusion dependent, with the exception of individuals I-1 from families 12, 25, 29, 35, II-1 from families 9, 21, 22, 27, 39, 45, and II-2 from family 37 who have thalassemia intermedia.

Hematological Studies

Blood samples were collected in EDTA and processed in Portugal within 8 hr. Hematological indices were determined by an automated cell counter. Electrophoretic hemoglobin separation was performed by isoelectrofocusing. When detected by the former procedure, HbF was quantified by the alkali denaturation method of Betke [14]. γ Chain typing in HbF was performed by HPLC [15]. LPLC was used to quantify HbA_2 [16]. The presence of HbS was confirmed by the sickling test.

DNA Preparation and Molecular Analysis

DNA was prepared from peripheral blood leukocytes by the salting-out procedure [17] followed by micro phenol–chloroform extraction. The haplotype of the β globin gene cluster was determined according to the previously described PCR-based assay and restriction enzyme analysis [18]. The following restriction sites of the RFLP haplotype were studied: *Hin*cII/ε, *Hin*dIII/Gγ and Aγ, *Hin*cII/ψβ and 3′ψβ, *Ava*II/β, and *Hin*fI/3′β. Haplotypes were named according to Antonarakis et al. [19]. Extended haplotypes included the *Xmn*I polymorphism at −158 bp of Gγ globin gene, and polymorphism of $(AT)_xT_y$ motif at −540 bp β globin gene. The chromosomes were divided into two classes according to their extended 5′ subhaplotype: class A, in which the X*mn*I site was present and class B, in which it was absent. AA^T, BB^T, and BA^T refer to the genotypes of β thalassemic carriers [1]. The configuration of $(AT)_xT_y$ alleles was determined by direct sequencing using the ABI Prism® Big Dye™ technology from Applied Biosystems. Structural analysis of α globin genes was performed by DNA amplification [20,21] for the most frequent α globin gene deletions in the Mediterranean population ($\alpha^{3.7}$ and $\alpha^{4.2kb}$) [22]. Absence of promoter mutations at Aγ globin gene was ascertained by DNA amplification and sequencing with primers R159-(5′TGAAACTGTGGTCTTTATGAAAATTG3′) and R161-(5′TGGCGTCTGGACTAGGAGCTTATT3′) and at δ globin gene with R117 (5′GGGCAAGTTAAGGGAATA3′) and R119 (5′GGAGAAGAGCAGGTAGGT3′), [23]. The δβ Sicilian deletion was investigated according to Craig et al. [24].

Characterization of Thalassemia Mutations

ARMS (Amplification Refractory Mutation System) was used to screen for the presence of the four most

frequent mutations in the Mediterranean area [$\beta^0$39 (C→T); β^+IVS1,nt110 (G→A); β^+IVS1,nt6 (T→C); β^0IVS1,nt1 (G→A)] [18]. Confirmation of the ARMS result was performed by restriction enzyme digestion as follows: *Mbo*I for β^+IVS1,nt110 (G→A) [25], *Bsa*bI for β^0IVS1,nt1 (G→A), *Mae*I for $\beta^0$39 (C→T), *Bsu36*I for β^0FsCD6 (−A), and S*faN*I for β^+IVS1,nt6 (T→C) [26]. Direct sequencing using ABI Prism® 3100 and ABI Prism® Big Dye™ technology from Applied Biosystems was used to detect other mutations.

Statistical Analysis

The Levene test of homogeneity of variances was used. When there was homogeneity of variances, the one-way ANOVA procedure was applied. As post-hoc test, the Student–Newman–Keuls multiple comparison procedure was followed, as well as the Kruskal–Wallis test and the Mann–Whitney test according to Glantz [27].

RESULTS

Hematological Data

The hematological and genetic data concerning 46 Moroccan families with at least one β thalassemia patient are summarized in Table I. All β thal carriers presented microcytosis and hypochromia. The HbA_2 levels were within the standard values ($HbA_2 \geq 2.6\%$). The level of HbF in heterozygotes older than 4 years of age ranged from trace quantities to 2.38 g/dl (Table I). The individual I-1 from family 11 has HbA_2 of 2.8 and an abnormally high HbF level, 17.9% of total hemoglobin, with a Gγ/Aγ ratio > 1.

Molecular Analysis in the α and β Globin Loci: Correlation With HbF

Analysis of the α globin locus revealed four families (families 4, 5, 21, and 32) with the α globin genotype ($-\alpha^{3.7}/\alpha\alpha$) and one individual from family 43 with $-\alpha^{3.7}/\alpha\alpha\alpha$. In the β globin locus, the phase of the different restriction fragment length polymorphisms (RFLP haplotype) was ascertained by family studies (Table I). Haplotypes III, IV, and IX were found in 42 independent chromosomes, normal or β thal, in linkage disequilibrium with *Xmn*I (+). As the same association had been previously observed in normal and in β thal chromosomes carrying the Mediterranean haplotype III, IV, or IX, the β thal chromosomes were classified as having 5′ subhaplotype class A [1]. The remaining chromosomes were associated with *Xmn*I (−) and classified as having 5′ subhaplotype class B. Gene sequencing of individual I-1 from family 19 revealed a polymorphism at position −369 C→G of Aγ globin gene (http://www.ncbi.nlm.nih.gov/SNP).

In this sample, only the $(AT)_7T_7$ allele at −540 of the β^A or β thal genes was found (Table I). The effect of gender, mutation type and the *Xmn*I allele on the expression status of HbF in Heterozygotes was investigated. No distinct HbF median values were observed between genders (Levene test, $P = 0.165$; one-way ANOVA, $P = 0.396$, Fig. 1A). The statistical analysis of the correlation HbF/β thal mutation shows that there is no homogeneity of variances (Kruskal–Wallis test, $P = 0.044$). The Mann–Whitney test shows that the β^0FsCD6(−A) group was statistically different from $\beta^0$39 and from β^0FsCD8(−AA) by a P value of 0.014 and 0.035, respectively. All the other groups of mutations were homogeneous (Fig. 1B).

In order to analyze a homogeneous group, individual I-1 from family 11 (HbF 2.38 g/dl) and α thal carriers were excluded. This way, HbF values in the heterozygotes range from traces to 0.96 g/dl. Comparison of HbF levels among each genotypic group AA^T, BB^T, and BA^T shows no statistically significant differences (Kruskal–Wallis, $P = 0.779$, data not shown).

DISCUSSION

β Thalassemia is a major genetic disease and a public health concern in some Mediterranean countries. In Morocco, as in the rest of Northern Africa, it has been selected by malaria and amplified by endogamy. The nonexistence of a national control program as well as the costs that it would imply prevent the existence of efficient therapeutic support to all the affected people, thus being a good mandatory prevention strategy.

The present study characterizes 86 independent β thalassemic chromosomes, from 46 unselected Moroccan families. Six major mutations: $\beta^0$39 (C→T), β^0FsCD8(−AA), β^+ IVS1,nt6 (T→C), β^0IVS1,nt1 (G→A), β^0FsCD6 (−A) and β^+-29 (A→G) cap site account for 75% of the β thal chromosomes studied. The mutation β^++20 (C→T) was found in one family, *in cis* with β^+IVS2,nt745 (C→G) as previously detected [28]. The heterogeneity of mutations in this population further extends to β^+polyA (T→C); $\beta^0$25bp del 3′IVS1; β^0IVS1,nt130 (G→A); β^0IVS2,nt1 (G→A); β^0IVS1,nt2 (T→C); $\beta^0$37 (G→A); and β^+-28 (A→G).

Until now, no haplotype data had been gathered for the Moroccan population, thus preventing population studies and prenatal counseling. Eight out of 9 Mediterranean haplotypes are retrieved in Moroccan β thal chromosomes, but a different pattern of predominant mutations is observed when compared to other Mediterranean countries [29]. The most abundant mutations are $\beta^0$39 (15.5%) and β^0fsCD8(−AA) (15.5%) (Table II). The latter opposes the rarity of β^+IVS1,nt110 (2%), in association with haplotype I, as in Algeria [30], Tunisia [31], and Portugal [18,32], supporting the idea of a recent introduction in the Moroccan genetic background. The third most frequent mutation is β^+IVS1,nt6 (T→C) (14%) in haplotypes VI and VII as described in Portugal [18,32,33], whereas in Algeria, Tunisia, and Egypt it is associated with haplotype VI [34,35].

TABLE I. Summary of Hematological and Genetic Data From 46 Moroccan Families With β-Thalassemia*

	Subject	Sex (age)	Haplotype[a]	Mutation	Hb (g/dl)	HbF (%)	HbF (g/dl)	HbA_2 (%)	α Genotype	*Xmn*I (−158Gγ)	$(AT)_xT_y$ *x*/*y*	$(AT)_xT_y$ *x*/*y*
Fam. 1	I-1	F	^{T}II/VIII	$\beta^0$39/βA	9.8	Traces	Traces	4.3	αα/αα	–/–	7/7	7/7
	II-1	M(6)	(II/II)^T	$\beta^0$39/$\beta^0$39	8.4	7.5	0.63	2.3	αα/αα	–/–	nd	nd
Fam. 2	I-1	F	^{T}V/I	β^0IVS1,nt1/βA	10.6	Traces	Traces	3.9	αα/αα	–/–	7/7	7/7
	II-1	M(8)	(V/V)^T	β^0IVS1,nt1/β^0IVS1,nt1	9.6	Traces	Traces	2.4	αα/αα	–/–	nd	nd
Fam. 3	I-1	F	^{T}II/VI	$\beta^0$39/βA	10.8	Traces	Traces	4.4	αα/αα	–/–	7/7	7/7
	II-1	M(5)	(II/II)^T	$\beta^0$39/$\beta^0$39	6.7	Traces	Traces	2.4	αα/αα	–/–	nd	nd
Fam. 4	I-1	M	^{T}V/I	β^0IVS1,nt1/βA	13.3	3.7	0.49	4.1	−α3.7/−α3.7	–/–	7/7	7/7
	II-1	M(6)	(V/V)^T	β^0IVS1,nt1/β^0IVS1,nt1	4.3	6.0	0.26	2.2	−α3.7/αα	–/–	nd	nd
Fam. 5	I-1	F(40)	^{T}III/VI	β^0FsCD6/βA	8.3	4.0	0.33	3.6	αα/αα	+/–	7/7	7/7
	I-2	M(45)	^{T}III/IX	β^0FsCD6/βA	13.3	3.7	0.49	5.1	−α3.7/αα	+/+	7/7	7/7
	II-1	F(1)	(III/III)^T	β^0FsCD6/β^0FsCD6	8.0	50.2	4.02	2.7	αα/αα	+/+	7/7	7/7
	II-2	F(5)	^{T}III/IX	β^0FsCD6/βA	11.8	5.2	0.61	4.7	αα/αα	+/+	7/7	7/7
Fam. 6	I-1	M	^{T}I/I	β^+−28/βA	14	Traces	Traces	4.2	αα/αα	–/–	7/7	7/7
	I-2	F	^{T}IX/VIII	$\beta^0$25bp del3′IVS1/βA	11.3	Traces	Traces	3.7	αα/αα	+/–	7/7	7/7
	II-1	M(6)	(I/IX)^T	β^+−28/$\beta^0$25bp del3′IVS1	10	16.2	1.62	2.3	αα/αα	–/+	7/7	7/7
	II-2	F	^{T}IX/I	$\beta^0$25bp del13′IVS1/βA	10.8	Traces	Traces	3.9	αα/αα	+/–	7/7	7/7
Fam. 7	I-1	M	^{T}V/I	β^0IVS1,nt1/βA	13.1	Traces	Traces	4.3	αα/αα	–/–	7/7	7/7
	II-1	M(12)	^{T}V/Benin^S	β^0IVS1,nt1/βS	7.2	6.9	0.49	4.7	αα/αα	–/–	nd	nd
Fam. 8	I-1	F	^{T}IV/VII	β^0FsCD8/βA	12	Traces	Traces	4.5	αα/αα	+/–	7/7	7/7
	II-1	M(5)	(IV/VI)^T	β^0FsCD8/β^0FsCD8	8.4	15.9	1.33	2.1	nd	+/+	nd	nd
Fam. 9	I-1	F	^{T}I/III	β^+polyA/βA	13.8	Traces	Traces	3.3	αα/αα	–/+	nd	nd
	II-1	F	(I/I)^T	β^+polyA/β^+polyA	8.5	72.1	6.12	2.1	αα/αα	–/–	nd	nd
Fam. 10	I-1	F(32)	^{S}Benin/III	βS/βA	12.3	1.7	0.21	4.1	αα/αα	–/+	nd	nd
	II-1	F(5)	^{S}Benin/IX^T	βS/β^0FsCD6	9.9	10.5	1.04	2.5	nd	–/+	nd	nd
	II-2	M(1)	nd	βS/βA	9.6	4.0	0.38	3.8	nd	nd	nd	nd
Fam. 11	I-1	F(35)	^{T}IV/II	β^0FsCD8/βA	13.3	17.9	2.38	2.8	αα/αα	+/–	7/7	7/7
	II-1	M(6)†	(IV/IV)^T	β^0FsCD8/β^0FsCD8	8.8	43.7	3.84	nd	nd	+/+	nd	nd
Fam. 12	I-1	F(40)	–	β^+−101/β^0IVS1,nt130	9.8	4	0.27	2.0	αα/αα	–/–	nd	nd
Fam. 13	I-1	F	^{T}V/IX	β^0IVS1,nt/βA	10.8	Traces	Traces	3.9	αα/αα	–/+	7/7	7/7
	II-1	F(13)	^{S}Benin/V^T	βS/β^0IVS1,nt1	8.7	10.6	0.92	6.9	αα/αα	–/–	nd	nd
Fam. 14	I-1	F	^{T}3black/?	β^+−29/βA	10.5	Traces	Traces	3.7	nd	–/–	7/7	7/7
	II-1	F(20)	(IX/3black)^T	β^0FsCD6/β^+−29	10.6	6.7	0.71	2.4	αα/αα	+/–	nd	nd
Fam. 15	I-1	F	^{T}IV/?	β^0FsCD8/βA	10.3	Traces	Traces	4.3	αα/αα	+/–	7/7	7/7
	II-1	F(16)	(IV/IV)^T	β^0FsCD8/β^0FsCD8	8.3	45	3.73	2.5	αα/αα	+/+	nd	nd
Fam. 16	I-1	F	^{T}II/I	$\beta^0$39/βA	10.5	Traces	Traces	4.0	αα/αα	–/–	7/7	7/7
	II-1	M(13)	(II/II)^T	$\beta^0$39/$\beta^0$39	9.4	Traces	Traces	2.5	αα/αα	–/–	nd	nd
Fam. 17	I-1	F	^{T}IX/IX	β^0IVS1,nt1/βA	10.5	Traces	Traces	3.9	αα/αα	+/+	7/7	7/7
	II-1	F(10)	(IX/II)^T	β^0IVS1,nt1/$\beta^0$39	7.0	26.2	1.83	2.3	αα/αα	+/–	nd	nd
Fam. 18	I-1	F(44)	^{T}VII/I	β^+IVS1,nt6/βA	12.3	Traces	Traces	2.7	nd	–/–	7/7	7/7
	II-1	M(7)	(VII/VII)^T	β^+IVS1,nt6/β^+IVS1,nt6	8.8	5.3	0.47	3.1	αα/αα	–/–	7/7	7/7
	II-2	F(9)	(VII/VII)^T	β^+IVS1,nt6/β^+IVS1,nt6	9.4	7.0	0.66	3.1	αα/αα	–/–	7/7	7/7
Fam. 19	I-1	F(45)	^{T}3black/4chinese	β^+−29/βA	12.4	7.8	0.96	4.2	αα/αα	–/–	7/7	7/7
	I-2	M(45)	^{T}I/nd	β^+IVS1,nt110/βA	12.8	5.1	0.65	3.7	αα/αα	–/–	7/7	7/7
	II-1	M(6)	(I/3black)^T	β^+IVS1,nt110/β^+−29	9.3	54	5.06	2.6	αα/αα	–/–	7/7	7/7
	II-2	M(9)	^{T}I/4chinese	β^+IVS1,nt110/βA	11.5	3.0	0.34	4.1	αα/αα	–/–	7/7	7/7
	II-3	M(4)	^{T}3black/nd	β^+−29/βA	10.7	6.1	0.65	4.4	αα/αα	–/–	7/7	7/7
Fam. 20	I-1	F(50)	^{T}IX/IX	β^0FsCD6/βA	10.8	4.7	0.51	4.3	αα/αα	+/+	7/7	7/7
	I-2	M(60)	^{T}IX/II	β^0FsCD6/βA	13.2	1.8	0.24	4.4	αα/αα	+/–	7/7	7/7
	II-1	F(7)	(IX/IX)^T	β^0FsCD6/β^0FsCD6	7.4	16.7	1.23	2.1	αα/αα	+/+	7/7	7/7
	II-2	F(23)	^{T}IX/II	β^0FsCD6/β	10.5	3.0	0.31	4.6	αα/αα	+/–	7/7	7/7
	II-3	F(4)	IX/II	βA/βA	15.6	2.8	0.43	2.3	αα/αα	+/–	7/7	7/7
Fam. 21	I-1	F(56)	^{T}VI/I	β^+IVS1,nt6/βA	13.0	1.5	0.19	3.1	αα/αα	–/–	7/7	7/7
	I-2	M(57)	^{T}V/I	β^0IVS1,nt1/βA	14.2	1.2	0.17	4.3	−α3.7/αα	–/–	7/7	7/7
	II-1	M(20)	(V/VI)^T	β^0IVS1,nt1/β^+IVS1,nt6	9.9	39	3.86	3.6	−α3.7/αα	–/–	7/7	7/7
	II-2	M(18)	^{T}V/I	β^0IVS1,nt1/βA	12.4	1.3	0.16	4.4	−α3.7/αα	–/–	7/7	7/7
	II-3	M(16)	I/I	βA/βA	13.1	0.5	0.06	2.6	nd	–/–	7/7	7/7
	II-4	M(10)	^{T}VI/I	β^+IVS1,nt6/βA	13.7	0.6	0.08	3.8	−α3.7/αα	–/–	7/7	7/7
	II-5	F(8)	^{T}VI/I	β^+IVS1,nt6/βA	11.7	1.3	0.15	3.3	−α3.7/αα	–/–	7/7	7/7
Fam. 22	I-1	F(48)	^{T}3black/II	β^+−29/βA	12.0	6.0	0.72	4.1	nd	–/–	7/7	7/7
	II-1	F(32)	(3black/3black)^T	β^+−29/β^+-29	8.4	58.4	4.9	4.0	αα/αα	–/–	nd	nd

TABLE I. Continued

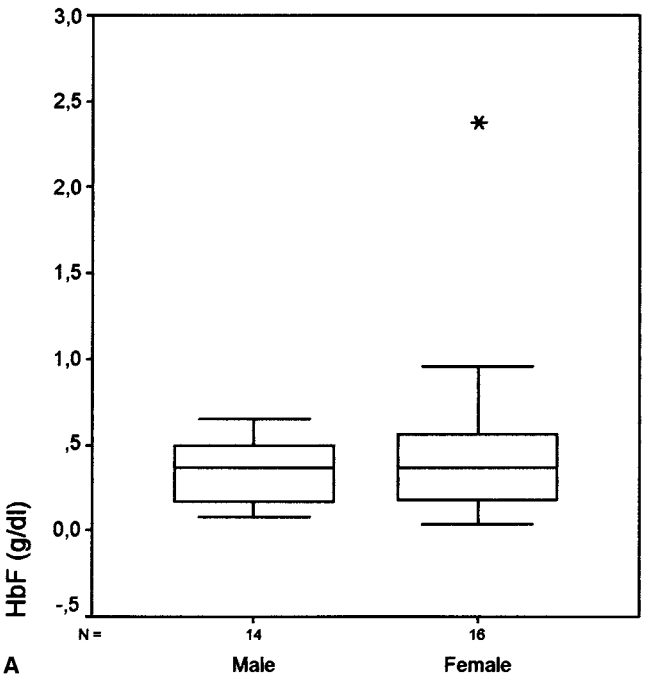
	Subject	Sex (age)	Haplotype[a]	Mutation	Hb (g/dl)	HbF (%)	HbF (g/dl)	HbA_2 (%)	α Genotype	*Xmn*I (−158Gγ)	$(AT)_xT_y$ *x/y*	$(AT)_xT_y$ *x/y*
Fam. 23	I-1	F(25)	^{T}II/I	$\beta^0$39/βA	11.3	3.1	0.35	4.3	nd	–/–	nd	nd
	II-1	M(5)	(II/II)^{T}	$\beta^0$39/$\beta^0$39	5.4	nd	nd	1.7	nd	–/–	nd	nd
Fam. 24	I-1	F(50)	^{T}IV/III	β^0FsCD8/βA	12.5	1.0	0.12	4.4	αα/αα	+/+	7/7	7/7
	II-1	F(14)	(VII/IV)^{T}	$\beta^0$37/β^0FsCD8	5.4	11.0	0.59	2.1	αα/αα	–/+	nd	nd
Fam. 25	I-1	F(58)	(VII/IV)^{T}	nd/β^0FsCD8	10.9	2.2	0.24	4.2	αα/αα	–/+	nd	nd
Fam. 26	I-1	F(30)	^{T}VII/I	β^+IVS1,nt6/βA	nd	nd	nd	nd	nd	–/–	7/7	7/7
	II-1	F(1)	(VII/VII)^{T}	β^+IVS1,nt6/β^+IVS1,nt6	nd	nd	nd	nd	nd	–/–	nd	nd
Fam. 27	I-1	F(28)	^{T}VI/II	β^+IVS1,nt6/βA	nd	nd	nd	nd	αα/αα	–/–	nd	nd
	II-1	M(2)	^{T}VI/II	β^+IVS1,nt6/Hb Newcastle	nd	nd	nd	nd	αα/αα	–/–	nd	nd
Fam. 28	I-1	F(51)	^{T}IV/II	β^0IVS1,nt1/βA	nd	nd	nd	nd	αα/αα	+/–	nd	nd
	II-1	F(12)	(IV/IV)^{T}	β^0IVS1,nt1/β^0IVS1,nt1	nd	nd	nd	nd	αα/αα	+/+	nd	nd
Fam. 29	I-1	M(34)	(VI/VI)^{T}	β^+IVS1,nt6/β^+IVS1,nt6	8.1	14.2	1.15	6.8	nd	–/–	nd	nd
Fam. 30	I-1	F(4)	(VII/VII)^{T}	β^+IVS1,nt6/β^+IVS1,nt6	5.8	3.5	0.2	2.2	nd	–/–	nd	nd
Fam. 31	I-1	M	^{T}I/V	β^+IVS1,nt110/βA	11.2	4.1	0.46	4.1	αα/αα	–/–	7/7	7/7
	II-1	F(7)	(I/III)^{T}	β^+IVS1,nt110/β^0IVS2,nt1	5.2	62.8	3.26	1.5	αα/αα	–/+	nd	nd
Fam. 32	I-1	F	IV/IV	β^0FsCD8/βA	11.6	4.4	0.51	4.0	αα/αα	+/+	7/7	7/7
	I-2	M	IV/VII	β^0FsCD8/βA	12.5	Traces	Traces	3.9	−α3.7/αα	+/–	7/7	7/7
	II-1	M(10)	IV/IV	β^0FsCD8/β^0FsCD8	8.3	3.9	0.32	2.3	nd	+/+	7/7	7/7
	II-2	F(13)	IV/IV	β^0FsCD8/β^0FsCD8	7.1	4.2	0.29	2.3	nd	+/+	7/7	7/7
Fam. 33	I-1	F	^{T}II/I	β^0IVS1,nt2/βA	9.7	3.2	0.31	3.7	αα/αα	–/–	7/7	7/7
	II-1	M(9)	(II/II)^{T}	β^0IVS1,nt2/β^0IVS1,nt2	4.3	6.1	0.26	2.4	αα/αα	–/–	nd	nd
Fam. 34	I-1	F	^{T}IX/I	β^0IVS1,nt2/βA	12.5	2.97	0.37	4.2	αα/αα	–/+	7/7	7/7
	II-1	M(12)	(I/IX)^{T}	$\beta^0$37/β^0IVS1,nt2	7.8	8.5	0.66	2.4	αα/αα	–/+	nd	nd
Fam. 35	I-1	F(28)	(IV/VI)^{T}	β^0FsCD8/β^+IVS1,nt6	8.1	Traces	Traces	2.7	nd	+/–	nd	nd
Fam. 36	I-1	M	^{T}VI/IX	β^+IVS1,nt6/βA	12	Traces	Traces	4.7	αα/αα	–/+	7/7	7/7
	II-1	M(14)	(IV/VI)^{T}	β^0CD8/β^+IVS1,nt6	9.2	14.9	1.37	3.4	αα/αα	+/–	nd	nd
Fam. 37	I-1	F	^{T}VII/?	β^+IVS2,nt745/βA	10.1	Traces	Traces	4.5	αα/αα	–/+	7/7	7/7
	II-1	F(8)	(VII/IV)^{T}	β^+IVS2,nt745/β^0FsCD8	9.6	15.9	1.52	2.8	αα/αα	+/–	nd	nd
	II-2	M(4)	(VII/IV)^{T}	β^+IVS2,nt745/β^0FsCD8	16.8	14.1	2.36	2.3	αα/αα	+/–	nd	nd
Fam. 38	I-1	F	^{T}VII/IX	β^0IVS1,nt6/βA	10.9	Traces	Traces	4.1	αα/αα	–/+	7/7	7/7
	II-1	F(1.5)	(VII/VII)^{T}	β^0IVS1,nt6/β^0IVS1,nt6	6.5	75	4.87	2.2	αα/αα	–/–	nd	nd
Fam. 39	I-1	F	^{T}IV/I	β^0FsCD8/βA	10	Traces	Traces	4.4	αα/αα	+/–	7/7	7/7
	II-1	M(17)	(IV/VI)^{T}	β^0FsCD8/β^+−29	9.7	30.7	2.97	2.6	αα/αα	+/–	nd	nd
Fam. 40	I-1	F	^{T}IX/IX	β^0FsCD6/βA	7.4	0.6	0.04	4	αα/αα	+/+	7/7	7/7
	II-1	M(8)	(IX/IX)^{T}	β^0FsCD6/β^0FsCD6	8.3	19.8	1.64	2.1	αα/αα	+/+	nd	nd
Fam. 41	I-1	F	–	β^+IVS1,nt6/βA	10.6	3.9	0.41	4.6	αα/αα	+/–	7/7	7/7
	II-1	F(7)	(IV/VII)^{T}	nd/β^+IVS1,nt6	7.9	42.1	3.32	2.4	αα/αα	+/–	nd	nd
Fam. 42	I-1	F	^{T}V/I	β^0IVS1,nt1/βA	12.1	3.7	0.45	4.8	αα/αα	–/–	7/7	7/7
	I-2	M	^{T}V/I	β^0IVS1,nt1/βA	13.3	4.5	0.6	4	αα/αα	–/–	7/7	7/7
	II-1	M(3)	(V/V)^{T}	β^0IVS1,nt1/β^0IVS1,nt1	7.6	2.7	0.2	2.3	αα/αα	–/–	nd	nd
Fam. 43	I-1	F	^{T}II/II	β^+−29/βA	10.8	Traces	Traces	4.0	−α3.7/ααα	–/–	7/7	7/7
	II-1	M(17)	(II/II)^{T}	$\beta^0$39/β^+−29	9.9	5.5	0.54	2.4	−α3.7/αα	–/–	nd	nd
Fam. 44	I-1	F	^{T}II/?	$\beta^0$39/βA	11.7	Traces	Traces	4.2	αα/αα	–/–	7/7	7/7
	I-2	M	^{T}II/I	$\beta^0$39/βA	11.6	Traces	Traces	4.9	αα/αα	–/–	7/7	7/7
	II-1	F(4)	(II/II)^{T}	$\beta^0$39/$\beta^0$39	6.4	55	3.52	2.0	αα/αα	–/–	7/7	7/7
Fam. 45	I-1	F	I/VII	nd	10.7	Traces	Traces	1.9	αα/αα	–/–	7/7	7/7
	II-1	M(8)	^{T}IX/I	β^0sD6/nd	9.4	Traces	Traces	4.6	αα/αα	+/–	nd	nd
Fam. 46	I-1	M	^{T}I/VIII	$\beta^0$39/βA	12.2	Traces	Traces	4.1	αα/αα	–/–		
	I-2	F	^{T}II/VIII	$\beta^0$39/βA	11.1	Traces	Traces	4.6	αα/αα	–/–		
	II-1	M(9)	(I/II)^{T}	$\beta^0$39/$\beta^0$39	12.9	66	8.5	2.2	αα/αα	–/–		
	II-2	F	(I/II)^{T}	$\beta^0$39/$\beta^0$39	8.2	54	4.42	1.2	αα/αα	–/–		

*Abbreviations: M, male; F, female; nd, not determined.

[a]Haplotype according to Antonarakis et al. (1985): ^{T}, β thal; ^{S}, βS; †dead; GγAγ = 0.25 (Fam. 19, I-1) and 1.9 (Fam. 11, I-1).

β^0IVS1,nt1 (G→A), found mostly in Berbers in Algeria associated with haplotypes I, III, V, IX, and A [36,37], is found in Morocco in haplotypes IV and V with a frequency of 13%. β^0FsCD6 (−A) in haplotypes III and IX, has a frequency similar to that of Algeria (haplotypes I, IX, and A) and Tunisia (haplotypes IX, A, and Va).

Fifty-six percent of the patients are homozygous for mutations and haplotypes, confirming the high degree of



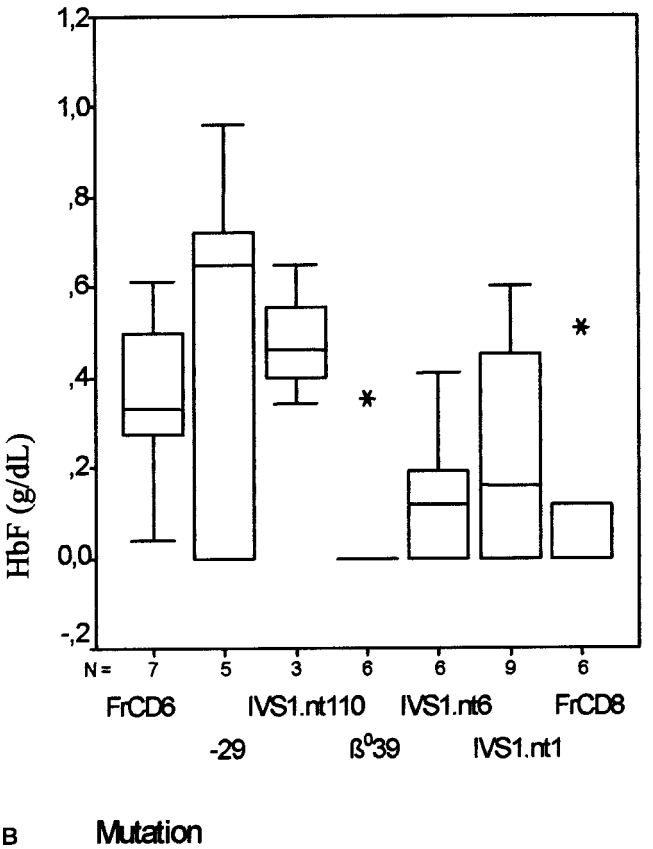


Fig. 1. Absolute fetal hemoglobin (HbF) levels represented by box plots. (A) Individuals are grouped by sex: 14 males, 16 females. (B) Individuals are categorized according to the mutation type: *N* = number of individuals.

TABLE II. Frequency and Haplotype Distribution of β Globin Gene Mutations

Mutation	No. of chromosomes	Frequency	Haplotypes
$\beta^0$39 (C→T)	14	15.5	I, II
β^0FsCD8 (−AA)	14	15.5	IV, VI
β^+IVS1,nt6 (T→C)	13	14	VI, VII
β^0IVS1,nt1 (G→A)	12	13	V, IV, IX
β^0 fsCD6 (−A)	9	10	IX, III
β^+−29 (A→G)	6	7	3black, II, VI
β^0IVS1,nt2 (T→C)	3	3	II, IX
$\beta^0$37 (G→A)	2	2	VII, I
β^+IVS1,nt110 (G→A)	2	2	I
β^0IVS1,nt130 (G→A)	1	1	–
β^+−101 (C→T)	1	1	–
β^0IVS2,nt1 (G→A)	1	1	III
β^+−28 (A→G)	1	1	I
β^+IVS2,nt745 (C→G)	1	1	VII
β^++20 (C→T)	1	1	VII
β^+polyA (T→C)	2	2	1
$\beta^0$25bp del 3′IVS1	1	1	IX
Hb Newcastle	1	1	II
βS	3	3	Benin
nd[a]	3	3	3
Total	90	100	–

[a]nd, not determined.

endogamy in this society as previously observed for Algeria and Tunisia. Homozygosity was also observed for less frequent or rare mutations in this population such as β^0IVS1,nt2 (T→C) found in American blacks and β^+polyA (T→C) in Middle East.

The individual I-1 from family 12 is a compound heterozygote β^0IVS1,nt130 (G→A)/β^+-101 (C→T) with a thalassemia intermedia phenotype [38], with an HbA_2 borderline (2%) and 4% HbF. The screening for mutations in the δ globin gene as well as for the most frequent α thal deletions was negative. However, it has not been excluded that another mutation in the α globin gene could be present causing the hematological phenotype.

In family 34, the β^0IVS1,nt2 (T→G) (Saudi Arabian mutation) is in haplotype IX, associated with $\beta^0$37 in haplotype I, as observed in Egypt [35].

An isolated individual (I-1, fam. 11), heterozygote for β^0FsCD8 (−AA) has HbF of 2.38 g/dl, presents a Gγ/Aγ >1 and a borderline level of Hb A_2. Although the screening for the Sicilian δβ deletion was negative and the α genotype had no alterations, a deletion covering δ and part of β gene cannot be excluded. Anomalies in the δ gene have not been screened due to lack of genomic material.

The β^+-29 mutation was found in three families (14, 19, and 22), associated with Orkin's haplotype 3 black and variable HbF levels as observed in the black population from America [39] and in one Chinese family [40,41]. Two families (39 and 43) present the β^+-29

mutation associated with the haplotype II and VI and traces of HbF.

Data from individual I-1, family 19, deserves further analysis: high HbF (0.96 g/dl), a Gγ/Aγ > 1, the -29 β promoter mutation, and the -369 C→G polymorphism in the Aγ promoter are observed. In silico analysis of the -369 Aγ polymorphism predicts a protein binding site for protein *sry* beta of *Drosophila melanogaster* (http://www.softberry.com), related to *sox,* a member of the HMG Box family of development-associated proteins [42]. The -369 Aγ polymorphism has been found linked to separate β globin cluster markers, associated with low HbF levels in β thal homozygotes, a situation not comparable to our study in heterozygotes [43]. Its possible role as modulator of expression of human globin genes [44] needs further investigation. This -369 Aγ polymorphism is commonly found in the Gγ gene [45], but its role in gene regulation has not yet been described. In the absence of experimental data on the regulatory function of the Gγ and Aγ polymorphisms per se or as linkage markers of HbF expression, one could speculate that the -369 Aγ polymorphism, in association with the reduction of β gene transcription by the TATA box mutation [46], possibly creates an imbalance of transcription factors, favoring an HbF elevation under the mild erythropoietic stress caused by β thalassemia heterozygous condition. Epidemiological studies of β thalassemia homozygotes, heterozygotes, and Hb Lepore heterozygotes [1,10,11], show the importance, for the regulation of fetal genes, of effector(s) located in both the adult and fetal domains of the cluster. The hypothesis of a regulatory mechanism based on transcription factors competition for the LCR cannot be excluded.

A review of the literature on HbF levels in heterozygous β thalassemia shows that the Moroccan population follows a similar distribution to that of the Portuguese [1]. No statistically significant correlation existed between sexes or mutation types and HbF levels. However, the β^0FsCD6 (−A) mutation exhibits statistically different behavior from the $\beta^0$39 and β^0FsCD8 (−AA) mutations (*P* values of 0.014 and 0.035, respectively).

The variability of HbF levels in this study was examined and related to the genetic markers previously identified in other populations [1,10,11]. All the individuals studied were homozygous for the allele 7/7 at −530 β globin gene. No differences between HbF levels and genotypes were found, contrary to previously observations in individuals of European descent [1,10,11]. Further studies will be required in order to explain the complex molecular mechanism of the HbF elevation in β thalassemia and more specifically in this heterozygous β thalassemic population of non-European descent.

Despite the heterogeneous spectrum of mutations, this evaluation allows the establishment of a prevention program by means of direct detection of the six predominant mutations, aiming at reducing the number of homozygote/double heterozygote births and preventing thalassemia major in Morocco.

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