Hemoglobin, 34(1):1–23, (2010)
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ISSN: 0363-0269 print/1532-432X online
DOI: 10.3109/03630260903571286

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REVIEW ARTICLE

HEMOGLOBINOPATHIES IN NORTH AFRICA: A REVIEW

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□ *Hemolytic anemias are very common diseases. Among these diseases, hemoglobinopathies are widely spread throughout the Mediterranean Basin, including North Africa (Tunisia, Algeria and Morocco). Their severity and disabling nature make them a major public health problem. This study includes our data on the Tunisian hemoglobinopathies together with all the reports concerning epidemiological, clinical and molecular aspects in Algerian and Moroccan populations. Investigation methods begin with the application of several techniques for hemoglobin (Hb) analyses [electrophoresis and isoelectric focusing (IEF), micro-chromatography assay] of anemic patients in various hospital departments. Molecular investigation by DNA analyses completes the hematological and biochemical studies using polymerase chain reaction (PCR) followed by enzymatic digestion and/or denaturing gradient gel electrophoresis (DGGE), single strand conformation polymorphism (SSCP) and sequencing. These methods offer screening for a large number of families affected by sickle cell disease and thalassemia. In Tunisia, Algeria, and Morocco, more than 45 mutations have been identified on the β-globin gene. The most common in Tunisia and in Algeria are codon 39 (C>T) and IVS-I-110 (G>A), which together account for more than 50% of all mutations. In Morocco, the predominant mutations are codon 39 and frameshift codon (FSC) 8 (–AA). The identification of molecular defects in the βgene contributes to the development of diagnostic tests (prenatal diagnosis), and gives us the opportunity to help many couples. Our studies of the haplotypes of the β^S, codon 39 and IVS-I-110 origins allowed the hypothesis of a Benin origin for β^S, a local North African origin for codon 39 and an Eastern Mediterranean origin for IVS-I-110. The analysis of polymorphisms associated with a moderate phenotype of β-thalassemia (β-thal) and sickle cell disease in North Africa has shown, in several cases, a strong association with some mutations and restriction fragment length polymorphisms (RFLP) haplotype IX on the β-globin locus and the −158 (C>T) polymorphism in 5′*

Received 15 May 2009; Accepted 11 October 2009.

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on the $^{G}\gamma$-globin gene. Finally, more knowledge on the regulation of the β-globin locus may contribute to the improvement of investigation, monitoring and treatment of hemoglobinopathies.

Keywords Hemoglobinopathies, Thalassemia, Sickle cell disease, Mutation, Haplotype

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INTRODUCTION

Hemoglobinopathies are defined by the presence of qualitative and/or quantitative abnormalities affecting the globin chains (1). To date, more than 700 abnormal β-globin gene hemoglobin (Hb) variants have been described (2,3). Qualitative abnormalities lead to the production of an abnormal structure of Hb. Among these variants, the best known are Hb S [β6(A3)Glu→Val] causing sickle cell disease, Hb C [β6(A3)Glu→Lys] and Hb E [β26(B8)Glu→Lys] (2,3), mostly because of the biological consequences they generate. However, the majority of variants are asymptomatic and therefore remain unknown. Hemoglobin quantitative abnormalities result from either reduced or absent synthesis of α- or β-globin chains. They define the thalassemias [α- and β-thalassemia (α- and β-thal), respectively)] For α-thalassemias, the common molecular defects are deletions involving either one, two, or three or the four alleles at the α locus (4). In contrast, β-thalassemias are predominantly the consequence of punctual mutations affecting a single β gene or both, resulting in a decrease (β^{+}-thal) or a total lack of synthesis of β-globin (β^{0}-thal) (1). Molecular abnormalities of regulatory regions on β or γ genes affecting gene expression result in silent thalassemias (β^{++}). They are the source of hereditary persistence of fetal Hb (HPFH) (5). Many forms are associated with qualitative and quantitative abnormalities of globin chains such as β^{S}/β^{thal} hemoglobinopathies or Hb E associated with α- or β-thalassemias (compound hemoglobinopathies).

Hemoglobinopathies are common all around the Mediterranean Basin. The Maghreb, with its geographical location (migration crossroad between sub-Saharan Africa, the Arab countries and Europe), its history and its socio-economic and cultural system, is one of the interesting geographic hot-spots. Moreover, the selective pressure of *Plasmodium falciparum* in regions endemic for malaria could have increased the frequencies of the β and α mutated genes (balanced selection) (6). Finally, the common endogamous system and consanguinity favor the increasing number of individuals affected. These pathologies are incapacitating by their severity and the lack of any cure so far. They represent a major public health problem. The fruitful collaboration of obstetricians and hematologists allowed the performance, in a few years, of a significant number of tests, with better reliability and increased security. Molecular analyses of the β-globin gene allowed the identification of numerous β-thal mutations. Many polymorphisms were found to be associated with some of these mutations. They helped us to understand the origins of these mutations.

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This study provides, for the first time, a thorough overview of α-, β-thal and Hb variants in the Maghreb countries. Knowledge of the molecular defects and haplotypes associated with these defects allows the development and improvement of diagnostic tests and the management of these diseases. In addition, it helps us to understand the origins and the migration schemes of these mutations in the Mediterranean area.

EPIDEMIOLOGICAL STUDY

Among the abnormal Hbs, sickle cell disease is by far the most frequent one. It affects mostly people from Africa, Madagascar, Reunion and Caribbean islands, Central America, the Mediterranean Basin and the Middle East (7). In Tunisia, the average frequency of the disease is 1.89% (8). It is between 0.8 to 3.5% in Algeria (9) and 1.2% in Morocco (10).

β-Thalassemias are frequently identified in subjects from the Mediterranean area (Italy, Sardinia, Sicily, Greece, North Africa), but they are also found in patients coming from Africa and Asia (Iran, India, Vietnam, Thailand) (11). In the Maghreb, β-thal reaches 2.21% in Tunisia (10) and almost the same frequency (1.5 to 3.0%) in Algeria (12,13) and Morocco (10). The α-thalassemias, however, are found mainly in populations of Southeast Asia (Cambodia, Laos, Myanmar, Thailand) (14) but also in the Mediterranean Basin and Central African countries (11). α-Thalassemias show an incidence of 4.8 to 5.48% in Tunisia (8) (7.38% for Hb Bart's (15), 9.0% in Algeria (12) and 2.2% in Morocco (10). However, the frequency of α-thal is probably underestimated: indeed, it remains difficult to identify it in adulthood by simple methods, especially when only one or two of the four α genes are affected. Today, population migrations and mixing favor a spread of these abnormalities which are found almost everywhere in the world. Some abnormal Hbs, once specific to ethnic groups, are now also found in other groups.

PHENOTYPICAL ANALYSIS

The identification of abnormal Hbs by phenotype analyses requires accurate information: ethnic origin of patients and their relatives, family history concerning hemoglobinopathies, clinical data and recent transfusion data. The diagnosis requires a recent complete blood count (CBC). Hemoglobin, mean corpuscular volume (MCV) and the number of red blood cells (RBC) must be interpreted according to the age of the patient (16). The examination of blood smears allows the recognition of any abnormalities of RBC: anisocytosis, microcytosis, polychromatosis, presence of target or sickled cells, basophilic stippling. Chromatographic methods allow the quantification of different fractions of Hb (Table 1). Anionic

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TABLE 1 Hematological Mean [± SD (Standard Deviation)] Data of a Tunisian Sample Composed of 30 Controls, 10 β-Thalassemia Major (β-TM), Seven β-Thalassemia Intermedia (β-TI), 21 Sickle Cell Patients (Hb S), and Six Compound Heterozygotes (β^S/β-thal)

Mutation	RBC (10^{12}/L)	MCV (fL)	Hb (g/dL)	Hb A (%)	Hb A_2 (%)	Hb F (%)	Hb S (%)
Controls ($n = 30$)	6.93 ± 0.25	87.70 ± 6.50	13.50 ±1.50	97.82 ± 0.17	2.18 ± 0.17	0.00	0.00
β-TM ($n = 10$)	2.68 ± 0.8	77.48 ± 7.94	5.98 ± 1,98	0.00	1.94 ± 0.35	98.60 ± 0.35	0.00
β-TI ($n = 7$)	2.72 ± 0.78	80.86 ± 3.88	7.54 ± 2.37	78.31 ± 11.15	1.68 ± 0.47	19.00 ±11.03	0.00
Hb S ($n = 21$)	2.59 ± 0.79	81.15 ± 8.77	6.75 ± 1.81	0.00	2.97 ± 0.95	5.16 ± 8.56	98.77 ± 8.72
β^S/β-thal ($n = 6$)	2.81 ± 0.93	75.26 ± 5.92	6.25 ± 1.70	0.00	3.23 ± 1.89	32.51 ± 7.79	63.73 ± 7.68

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exchanger micro-chromatography gives a rapid measurement of the Hb A_2 level (17): a rate exceeding 3.5% suggests a heterozygous β-thal. High performance liquid chromatography (HPLC) makes possible the identification and quantification of most Hbs (18). Other complementary tests are sometimes necessary such as the determination of Hb F by measuring the resistance to alkaline denaturation and a set of other tests specific to each case. These include the sickling test, Itano test, isopropanol instability test, cresyl blue instability test and Kleihauer-Betke test (19).

Among the electrophoretic methods, electrophoresis at alkaline pH on cellulose acetate or agar is one of the basic tests for the detection of hemoglobinopathies (20). Isoelectric focusing allows the detection of all normal and abnormal Hbs (19).

SCREENING AND GENETIC COUNSELING

Programs of systematic screening of hemoglobinopathies by phenotype analysis were established for subjects at risk. The detection of heterozygotes, asymptomatic in most cases, allowed us to identify couples at risk and eventually to propose genetic counseling. In Tunisia, the first step of genetic counseling for hemoglobinopathies was started in 1986 (21). In November 2006, a world congress was held in Marrakech, Morocco, on the theme "Strengthening of neonatal screening in North Africa and the Middle East." This congress was designed to establish a national screening program in North African countries, and the Middle East in general, and in Morocco in particular, for genetic diseases often detected in newborns including hemoglobinopathies.

The aim of genetic counseling is to help families carrying mutant alleles. It helps the medical staff for the potential screening of individuals at risk and to propose the antenatal diagnosis. The study requires informed and written consent from the patient(s) and family members. First, the investigation of the abnormalities responsible for the pathology is performed, followed by confirmation of the diagnosis and then an appropriate management strategy is proposed.

MOLECULAR ANALYSIS

The phenotype, even with a complete family investigation, sometimes needs a genotype study to detect the mutation allowing unambiguous diagnosis confirmation. Such an analysis requires a maximum of clinical and biological information and, if possible, samples of parents and relatives of the index cases.

Venous blood was collected in EDTA for DNA extraction by the phenol/chloroform and the salting extraction methods. Molecular hybridization, polymerase chain reaction (PCR) and sequencing of the β-globin gene are used in Tunisia, some in Algeria, and to a lesser extent, in Morocco. Early

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investigations used the Southern blot method for the restriction fragment length polymorphism (RFLP) studies in Tunisia (22,23), in Algeria (24–26) and in Morocco (13). The dot-blot method was used in Tunisia (27–29). The PCR-based methods for the identification of mutations have been widely used in Tunisia [PCR, allele-specific PCR, gap-PCR, RFLP-PCR, amplification refractory mutant system (ARMS)-PCR] (15,28–37), in Algeria (PCR, AS PCR, RFLP-PCR) (24,25,38–41) and in Morocco (PCR, AS PCR, RFLP-PCR) (13,42). Analysis of known and unknown mutations can be done by electrophoresis of PCR products on polyacrylamide gels in non denaturing [single-strand conformational polymorphisms (SSCP)] and denaturing conditions [denaturing gradient gel electrophoresis (DGGE)]. This method is employed in Tunisia (29,32,34) together with sequencing for the identification of the globin gene mutations (15,34,37,43–45).

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IDENTIFICATION OF MUTATIONS

The investigations on thalassemias in Tunisia, Algeria and Morocco contributed to establishing the spectrum of mutations in the three countries. The total number of β-thal mutations identified in Tunisia was 24 (Table 2) (13,29,33,42,44,46–49). The codon 39 (C>T) and IVS-I-110 (G>A) mutations are largely predominant (from 54 to 70% of β-thalassemic mutations). Twenty-six mutations are described in Algeria (Table 2). The most frequent mutations are codon 39, IVS-I-110 (equal frequency), codon 6 (–A) and IVS-I-1 (G>A) which account for about 80% of the independent chromosomes (Table 2). In Morocco, recent studies showed a spectrum of 26 different β-thalassemic mutations. The first six [codon 39, codon 8 (–AA), –29 (A>G), codon 6, IVS-I-6 (T>C) and IVS-I-1] represent 60 to 75% of the β-thalassemic Moroccan chromosomes (Table 2).

In addition to all these mutations observed in cases of β^0- or β^+-thal, 22 Hb variants were described in the three countries (Table 3) (45,48,50–67). Three of them are found in all the Maghreb countries: Hb G-Philadelphia [α68(E17)Asn→Lys], Hb D-Punjab or Hb D-Los Angeles [β121(GH4)Glu→Gln] and Hb O-Arab [β121(GH4)Glu→Lys] (51,57). Concerning α-thal, 12 mutations have been detected (Table 4) (15,31,68–71). Not surprisingly, two of them are widespread, *i.e.,* $-\alpha^{3.7}$ and $--^{MED-I}$. This reduced heterogeneity could be explained by the underestimation of these defects which cannot be observed in adult life. Together, thalassemia disorders and Hb variants account for 81 mutations or variants in the three Maghreb countries.

All these genetic studies show that Maghreb populations display some heterogeneity, especially between Algeria, Tunisia and Morocco. This heterogeneity is due to new mutational events or gene flow due to human migrations, and probably reflects some differences in the historical pattern of migration and colonization. The three populations show a large spectrum of

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TABLE 2 Spectrum of Relative Frequencies (%) of β-Thalassemia Mutations in Tunisia, Algeria and Morocco

Mutation	Type	Origin	Tunisia			Algeria		Morocco	
References			44	29	33	47	46	42	48
Codon 39, C>T	β^0	Mediterranean	48.76	43.78	49.00	25.90	27.60	26.58	26.20
IVS-I-110, G>A	β^+	Mediterranean	15.70	10.81	21.00	26.40	24.70	4.70	3.21
Codon 8, –AA	β^0	East European	–	0.54	0.20	0.90	–	13.91	9.63
Codon 6, –A	β^0	North African	1.65	7.02	2.60	12.90	17.10	5.70	13.37
IVS-I-6, T>C	β^+	West Mediterranean	2.48	0.54	0.60	6.20	3.30	3.16	13.90
IVS-I-1, G>A	β^0	Asian	3.31	4.32	4.50	9.10	11.70	5.06	8.56
Codon 5, –CT	β^0	Greek	–	–	0.40	–	–	1.27	–
Codon 44, –C	β^0	Kurdish Jews	1.65	1.62	3.80	–	–	–	–
IVS-I-2, T>C	β^0	Algerian; Russian	–	0.54	–	0.90	3.30	5.06	2.14
IVS-I-2, T>G	β^0	Tunisian	19.83	0.54	3.00	–	–	3.16	0.53
–30, T>A	β^+	Turkish	–	–	0.80	–	0.40	–	–
–29, A>G	β^+	Black	–	1.08	–	1.40	3.80	6.33	4.28
IVS-II-2, T>A	β^+/β^{0a}	Turkish	–	–	–	–	–	–	3.20
IVS-II-849, A>C	β^0	Black	–	4.32	0.04	–	–	–	–
IVS-II-745, C>G	β^+	Mediterranean	–	2.16	2.60	–	0.90	7.60	0.53
Codon 16, –C	β^0	Southeast Asian	–	–	–	–	–	–	2.60
Codon 30, G>C (Hb Kairouan)	β^+	Tunisian	2.48	3.24	3.20	1.40	0.90	–	0.53
IVS-I-5, G>A	β^+	West Mediterranean	3.31	–	1.50	–	0.90	–	–
IVS-I-5, G>C	β^+	Asian	–	3.24	1.00	1.90	0.40	–	–
IVS-I-5, G>T	β^+	Mediterranean	–	2.16	–	0.90	–	–	0.53
Codon 37, G>A	β^0	Middle East	–	1.08	–	–	–	3.16	1.07
–28, A>C	β^+	Kurdish Jews	–	–	–	–	–	1.90	–
IVS-II-1, G>A	β^0	Black; Mediterranean	–	1.62	0.60	0.90	–	2.50	–
–87, C>G	β^+	East Mediterranean	–	–	1.70	–	–	–	–
9 bp deletion/31 bp insertion	β^0	Algerian	–	–	–	0.40	–	–	–
Cap +20, C>T	β^+	Bulgarian; Moroccan	–	–	–	–	–	–	1.07
25 bp deletion 3′ IVS-I	β^0	Middle East	–	–	–	–	–	1.27	0.53
Codon 27, G>T (Hb Knossos)	β^+	Mediterranean	–	–	–	–	0.40	–	–
IVS-I-2, T>A	β^0	Algerian	–	–	–	–	1.30	–	–
IVS-II-726, A>G	β^+/β^{0a}	Moroccan	–	–	–	–	–	1.27	–
IVS-II-843, T>G	β^+	Algerian	–	–	–	0.90	0.40	–	–
IVS-II-848, C>A	β^+	Mediterranean	0.83	–	0.40	3.80	0.40	–	–
Hb Lepore-WB, δ87/βIVS-II-8[b]	β^+	Worldwide	–	–	–	0.40	0.40	–	–
–101, C>T (silent)	β^{++}	East Mediterranean	–	–	–	0.40	–	–	0.53
–90, C>T	β^+	Portuguese	–	–	–	0.40	–	–	–
–190, G>A	β^+	Moroccan	–	–	–	–	–	0.63	–
IVS-I-128, T>G	β^+	Southeast Asian	–	–	–	0.40	–	–	–
IVS-I-130, G>A	β^+	East Mediterranean	–	–	–	–	–	–	0.53
IVS-I-116, T>G	β^0	East Mediterranean	–	0.54	–	–	–	–	–
Codon 47, +A	β^0	Surinamese	–	–	–	0.90	–	–	–

(*Continued*)

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TABLE 2 (*Continued*)

Mutation	Type	Origin	Tunisia			Algeria		Morocco	
Poly A, AA*T*AAA>AA*C*AAA	β^+	Black; Turkish	–	–	–	–	–	–	1.07
Poly A, AATAA*A*>AATAA*G*	β^+	East Mediterranean	–	–	–	0.40	–	–	–
Codon 24, T>A	β^+	Japanese	–	–	–	–	–	0.63	–
Codons 25/26, +T	β^0	Tunisian	–	–	0.60	–	–	–	–
Unidentified			0.00	10.81	2.30	1.90	2.10	2.50	4.20
Others			–	–	–	–	–	2.50	–
Total number of chromosomes			121	185	475	208	239	158	187

Only sporadic studies have been done on the Tunisian poly A (AA*T*AAA>AA*A*AAA) (β^+) and codon 9, +TA (β^0) (49), and Moroccan −56, G>C (β^+/β^0) (13) mutations; no information concerning their frequency is available.

[a]β^+/β^0: No functional test was done.

[b]Hb Lepore-WB, δ87/βIVS-II-8: Hb Lepore-Washington-Boston.

mutations. Indeed, the Maghreb countries, by their privileged geographical position and diverse ethnic origins of their populations, represent an interesting area for the study of Hb disorders. Moreover, the high level of consanguinity, more particularly in rural areas where the frequency of marriages between first cousins is as high as 25–30% due to cultural traditions, increases considerably the probability of genetic disorders.

APPLICATION TO PRENATAL DIAGNOSIS

The fact that genetic technology makes the identification of molecular defects easier improves genetic counseling. The methods used in the antenatal period are different depending on whether it is diagnostic or screening. Diagnostic methods are based on samples of amniotic fluid (amniocentesis), fetal cells (choriocentesis), or fetal blood (cordocentesis). Prenatal diagnosis is proposed, with information and written consent, to couples having already given birth to an affected child, and therefore, at high risk.

Prenatal diagnosis consisted first of looking for an association between mutations in the β-globin gene and RFLP haplotypes on the β-globin locus. The assays began in Algeria in 1983 (72) and then in Tunisia in 1986 (21). Today, the combination of molecular biology techniques, such as ARMS-PCR, DGGE and sequencing, allows prenatal diagnosis in a rapid, reliable and inexpensive way, more particularly in families with an index case. Nowadays, this type of diagnostic test is commonly done in Tunisia (8,44,45), in Algeria (41,47) and in Morocco (42,48).

Preimplantation diagnosis (PGD), in the case of reproduction by in vitro fertilization (IVF) is beneficial for families who have an history of

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TABLE 3 Hemoglobin Variants Described in the Maghreb Countries

Variant name and substitution	Tunisia	Algeria	Morocco	Origin	Refs.
Dunn [α6(A4)Asp→Asn, α2 or α1]			X	Black; Indian; Moroccan	57
Boumerdés [α37(C2)Pro→Arg, α2 or α1]		X		Algerian	55
Montgomery [α48(CE6)Leu→Arg, α2]	X			Black; Chinese	58
G-Philadelphia [α68(E17)Asn→Lys, α2 or α1]	X	X	X	Black; Italian; Chinese	51
Loire [α88(F9)Ala→Ser, α2 or α1]		X		Algerian; French	56
Setif [α94(G1)Asp→Tyr, α1]		X		Algerian; Lybian; Iranian; Saudi Arabian	50
Melusine [α114(GH2)Pro→Ser, α2 or α1]		X		Algerian	64
Tunis-Bizerte [α129(H12)Leu→Pro, α1]	X			Tunisian	65
D-Ouled Rabah [β19(B1)Asn→Lys]		X		Tuareg; Algerian	63
D-Iran [β22(B4)Glu→Gln]	X	X		Iranian; Pakistani; Italian	62,63
Athens-GA [β40(C6)Arg→Lys]	X			Caucasian (SE USA)	60
Bab-Saadoun [β48(CD7)Leu→Pro]	X			Tunisian	61
Kenitra [β69(E13)Gly→Arg]			X	Moroccan	54
Newcastle [β92(F8)His→Pro]			X	Russian; Moroccan	48
Camperdown [β104(G6)Arg→Ser]	X			Maltese	53
D-Punjab [β121(GH4)Glu→Gln]	X	X	X	Southeast Asian; European	51
O-Arab [β121(GH4)Glu→Lys]	X	X	X	Arabian; Egyptian; Black; African American	51
Tunis [β124(H2)Pro→Ser]	X			Tunisian	59
F-Ouled Rabah [$^{G}\gamma$19(B1)Asn→Lys]			X	Algerian; Moroccan	66
F-Sardinia [$^{A}\gamma$75(E19)Ile→Thr]	X			Nearly worldwide	52
A_2-Pasteur-Tunis [δ59(E3)Lys→Asn]	X			Tunisian	45
Casablanca [β65(E9)Lys→Met; β122(GH5)Phe→Leu]			X	Moroccan	67

genetic disease. It allows early detection of a molecular defect and avoids the mother the trauma experienced in diagnosis during pregnancy. However, PGD is not yet practicable in the Maghreb medical centers. Only three centers in Saudi Arabia display such a PGD technique (73) in the Arab World. The sophisticated technology and tests are so expensive that this program is restricted to economically advantaged countries.

ANALYSIS OF HAPLOTYPE POLYMORPHISMS

Restriction Fragment Length Polymorphism and Single Nucleotide Polymorphism Haplotypes

The haplotype polymorphism analysis is of crucial importance in understanding the origin of mutations and for prenatal diagnosis of some

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TABLE 4 α-Gene Mutations Observed in Tunisia, Algeria and Morocco

		Tunisia			Algeria	Morocco
Mutation	Origin	Refs.	529 Cord blood samples %	304 Cord blood samples %	153 blood donors %	Refs.
$-\alpha^{3.7}$	Worldwide	68	1.13	3.45	2.9	71
$\alpha^{\text{Hph I}}\alpha$ [IVS-I-2 (–5 nt) (α2)]	Mediterranean		0.47	0.33	0.33	
$--^{\text{MED I}}$	Mediterranean	31	0.20	–	0.30	
$\alpha^{\text{T-Saudi}}\alpha$ Poly A, (AATAA*A*>AATAA*G*) (α2)	Arabian		0.94	0.66	–	
$-(\alpha)^{20.5}$	Mediterranean		–	–	0.30	
Codon 23, *G*AG>*T*AG (α2)	Tunisian		0.10	–	–	
$\alpha^{\text{Nco I}}\alpha$ [codon 1, ATG>GTG (α2)]	Italian		–	–	0.60	
Hb Groene Hart [codon 119, CCT>TCT (α1)]	Moroccan; Italian	31	0.10	–	–	71
$-\alpha^{4.2}$	Worldwide		–	0.33	0.16	
+6 (C>G) (α2)	Tunisian		0.10	–	–	
African polymorphisms [7238 G>TCGGCCC and 7174 T>G (α2)]	Tunisian; Surinamese		–	0.99	–	
+14 (G>C) (α2)	Tunisian		–	0.66	–	
References		31,68	15	69	70	71

hemoglobinopathies. Many studies have been done on the analysis of haplotypes associated with sickle cell disease and β-thal. These studies began with analysis of *Hpa*I RFLP haplotypes associated with sickle cell anemia (74). A few years later, haplotypes encompassing a set of polymorphic restriction endonucleases sites along the β-globin genes locus (Figure 1) were used (75). In Tunisia, the RFLP haplotypes have revealed an association between the typically Tunisian IVS-I-2 (T>G) β-thal mutation and haplotype IX (76). Other studies confirmed this result and showed associations of the codon 30 (G>C) mutation with haplotype I, and of the codons 25/26 (+T) mutation with haplotypes I and IX (35). In Algeria, many studies on RFLP haplotypes have been done, either for prenatal diagnosis or for the search of mutation origins (26,46,47,50,77–79). Restriction fragment length polymorphisms are also used in Moroccan population (13,42,48,80). All RFLP haplotypes observed in the three countries are summarized in Table 5 (22,26,35,42,46,76,77,81–86). More recently, studies have concerned other types of polymorphisms, the sequence polymorphisms in the region upstream of the β-globin gene which includes nine single nucleotide polymorphism (SNP) sites and an (AT)xTy composite microsatellite (Figure 1). We will call them SNP haplotypes. These polymorphisms were investigated for the first time in North Africa in the Algerian population (41,81) and then in the Tunisian population (32). In the Moroccan population, analysis was

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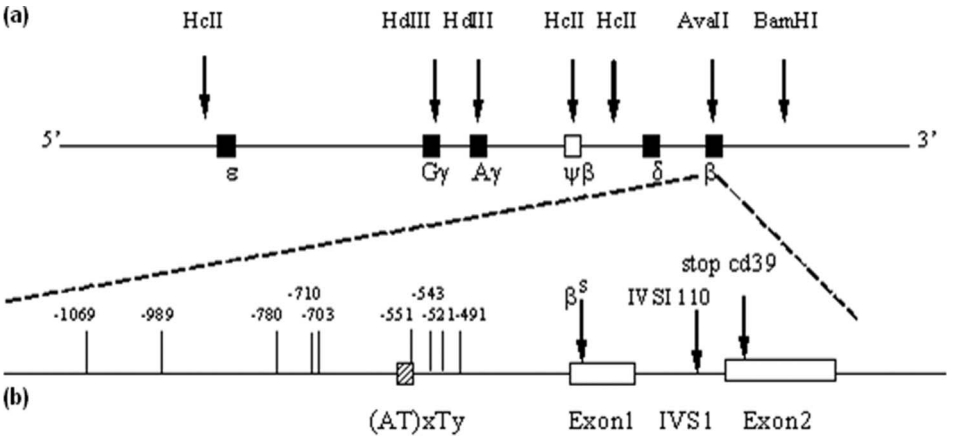

FIGURE 1 Map of the β-globin gene cluster and polymorphic sites used for RFLP haplotypes (a) and SNP haplotypes (b).

restricted to the (AT)xTy microsatellite (48,80). The SNP haplotypes observed in Tunisia and Algeria are shown in Table 5 (32,41,81,84,86). All these haplotypes have long been used for prenatal diagnosis and for the search of the origin and spread of the sickle cell and some β-thal mutations.

The C>T polymorphism at position −158 5′ of the $^{G}\gamma$ gene corresponding to a restriction site by the *Xmn*I enzyme was also widely used. This polymorphism is of crucial interest because of its suspected association with the moderate phenotype of β-thalassemic mutations. The first investigations of this polymorphism in North Africa have been conducted in the Algerian population (87), then in the Moroccan (80) and the Tunisian populations (unpublished data).

Origin of β^S and Common β-Thalassemia (IVS-I-110, codon 39 and IVS-I-1) Mutations in the Maghreb Countries

The origin of the β^S mutation has been widely studied. The last studies involving five RFLP and SNP haplotypes show that this mutation occurred five times independently in five regions of the world (multicentric origin), namely Benin, Senegal, Cameroon, Bantu and Saudi Arabian-Indian haplotypes (77,81). Tunisian β^S alleles are associated with the Benin haplotype according to RFLP (82), dot-blot (27) and SNP analyses (32). In Algeria, this mutation also appears to have a single Benin origin (77), as in Morocco and in Egypt, confirming a common genetic background for all the North African β^S alleles. Evidence suggests that during the Stone Age, the carriers of this allele traveled from Central West Africa across the then-fertile Sahara to the North. The presence of malaria among agricultural settlements in North Africa favored the Hb S gene. Later, as the Sahara began to dry up, there was a surge of migration away from the desert in all directions, spreading

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TABLE 5 Restriction Fragment Length Polymorphism and Single Nucleotide Polymorphism Haplotypes Associated with the Most Frequent β-Thalassemia Mutations in the Western and Eastern Mediterranean Area

	Morocco			Algeria						Tunisia					
Mutation	RFLP	%	Refs.	RFLP	%	Refs.	SNP	%	Refs.	RFLP	%	Refs.	SNP	%	Refs.
β6, A>T	Benin	–	42	Benin	100.0	77	Benin	100.0	81	Benin	94.0	82	Benin	97.0	32
										Atypical	4.0		Atypical		
										Benin	100.0	22		3.0	
Codon 39, C>T	I	–	42	I	33.7	26	HTR	63.6	41	I	14.3	76	HT1	87.0	a
	II	–		II	63.9		HT1	27.4		II	42.8		HTR		
	Nd	–		*	1.2		HT10	4.5		A	7.15		HT1	8.0	
				IX	1.2		HT11	4.5		5a	14.3		HTH	0.3	
										Nc	7.15			5.0	
										Nd	14.3			0.1	
														5.0	
IVS-I-110, G>A	I	–	42	–	–	–	HT1	70.0	41	I	100.0	76	HT1	86.0	a
	II	–					HT2	30.0					HT2	14.0	
IVS-I-2, T>G	IX	–	42	–	–	–	–	–	–	IX	100.0	35,76	HTR	10.0	a
										*Xmn*I	100.0	a		0.0	

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	Turkey						Lebanon					
Mutation	RFLP	%	Refs.	SNP	%	Refs.	RFLP	%	Refs.	SNP	%	Refs.
β6, A>T	–	–	–	–	–	–	Benin	73.0	85	–	–	–
							Cameroon	15.0				
							Saudi Arabian-Indian	10.0				
							Senegal	2.0				
Codon 39, C>T	II	50.0	83	HT1	55.0	84	II	66.7	86	HT1	10.0	86
	IV	50.0		HTR	45.0		I	33.3		–	0.0	
IVS-I-110, G>A	I	93.1	83	HT1	88.0	84	I	93.2	86	HT1	76.0	86
	II	4.1		HTR	5.0		5′-12	5.4		HTR	24.0	
	IX	1.4		HT3	3.5		II	1.0				
	IV	1.4		HT4	2.0							
				HT5	0.5							
				HT7	1.0							
IVS-I-2, T>G	–	–	–	–	–	–	–	–	–	–	–	–

The RFLP haplotypes: Benin [− − − − + + +]; I [+ − − − − + +]; II [− + + − + + +]; IV [− + − + + − +]; IX [− + − + + + +]; * [− + + − + + −]; A [− − − − − + +]; 5a [− + + − − + +]; Nc [+ + + − − + +]; Nd [− − − − + + +]; 5′–12 [− − − + + + +]. SNP haplotypes: –1069, –989, –780, –710, –703, –551, –543, –521, –491, (AT)x-Ty. Benin: AGATTTTCC 8-4; HT1: ACATTTCCA 7-7; HT2: ACATCCCCA 9-5; HT3: GCATCCCCA 11-3; HT4: GCATCCCCA 7-7; HT5: GCATCTCCA 7-7; HTY: ACATTCCCA 7-7; HT10: GCA TTTCCA 11-3; HT11: GGATTCCCA 11-3; HTR: GCATTTCCA 7-7; HTH: ACATTCCCA 9-5; HTI: GGATTTCCA 8-5.

[a]Haj Khelil et al., unpublished data.

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the gene further (78). However, all approaches used to estimate the age of the β^S mutation suggest that it arose about 3,000 years ago (88). Until now, there has been no evidence of a more ancient presence of this mutation. Its introduction in North Africa is probably more recent and due to the forced migrations from black Africa through the slavery roads and/or to the continuous influx of sub-Saharan Africans through the caravan routes (46).

For β-thal mutations, investigations have been conducted in the Algerian and Tunisian populations to support the hypotheses on the origin and spread of the most frequent β-thal mutations in these two countries (codon 39 and IVSI-110). The results were compared with those from other countries in the Mediterranean Basin in relation to the geographical, anthropological and historical background of this region. The analysis of RFLP and SNP haplotypes could support the hypothesis of a local origin of the codon 39 mutation in North Africa. Indeed, this mutation is predominant in Tunisia, Algeria and Morocco (8,42,47). It is much more common in the western part than in the eastern part of the Mediterranean Basin (Figure 2). Moreover, it shows high haplotype diversity: four RFLP haplotypes (46) and four SNP haplotypes (41) are associated with this mutation in Algeria. The same level of diversity has been observed in Tunisia for both RFLP haplotypes (76) and SNP haplotypes (unpublished data) (Table 5). For the IVS-I-110 mutation, the comparison of the data in Mediterranean countries strengthens the hypothesis previously proposed as a unique occurrence in the Eastern Mediterranean Basin (Anatolia) during the Neolithic period. Turkey showed the highest haplotype diversity for this mutation associated to six SNP haplotypes (84) (Table 5). Moreover, contrary to the codon 39 mutation, the IVS-I-110 is more common in the eastern Mediterranean region (Figure 2).

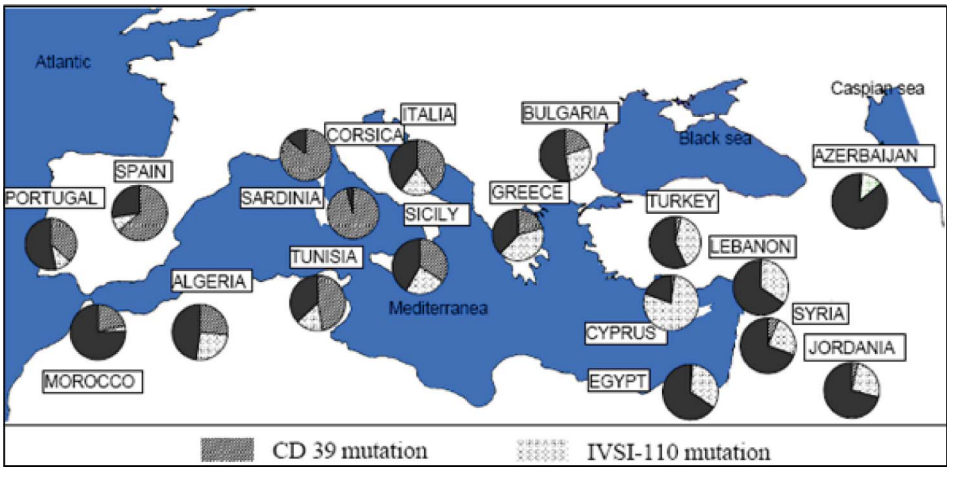

FIGURE 2 Distribution of the codon 39 and IVS-I-110 mutations in the Mediterranean Basin.

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This mutation could have been introduced into North Africa (Tunisia and Algeria) during the Ottoman rule in the 17th century. The fact that this mutation is the most frequent in Algeria, the second in Tunisia and the seventh in Morocco (infrequent), confirms this hypothesis. Indeed, the Ottoman occupation did not reach Morocco. Its limited introduction in this country would be the result of sporadic Algerian migrations. The results of haplotype analyses argue in favor of this hypothesis. Indeed, a single RFLP haplotype (I) was found in Tunisia (76). Two haplotypes (I and II) were found in Algeria (26,46). The predominant haplotype (I) is also described in Tunisia (92%). These two haplotypes are the most frequent of the four haplotypes found in Turkey (I, II, IV and IX) (83) (Table 5). These findings were confirmed by SNP haplotype analysis in Algeria (41) and in Tunisia (unpublished data) showing the association of the IVS-I-110 mutation with the same haplotypes: HT1 and HT2. HT1 is by far the most frequent haplotype as found previously in Turkey. The strong linkage disequilibrium between this haplotype and the IVS-I-110 mutation observed in Turkey, Algeria, and Tunisia strengthens the hypothesis of an East Mediterranean origin of this mutation in North Africa.

If we compare all the data, we notice important differences in the proportion of frequent mutations such as codon 39, IVS-I-110 and IVS-I-1. This probably reflects an unequal distribution of common Mediterranean mutations throughout the Maghreb. The IVS-I-110 mutation is predominant in the western part of Tunisia and in northern Algeria. This fits well with an Ottoman importation. This mutation is rare in Morocco which was never occupied by the Ottomans. Codon 39 is largely predominant in Tunisia, in Morocco and in the north-western part of Algeria. Codon 39 is likely to have an occidental and ancient origin (chromosomes are recombined in one-third of the cases) (Table 5). It could have been introduced into the Maghreb during the Roman period through Italy (41%) and Spain (35%) (89). The Roman Empire covered a large part of the Mediterranean area until the 5th century BC when the Byzantine reconquest occurred. The IVS-I-1 mutation is found mostly in the central region of Algeria and in Morocco. But it reaches its highest frequency in the Czech Republic and in Hungary (45 and 31%, respectively) (89). The lack of information concerning RFLP and SNP haplotypes in central European populations did not give us the opportunity to discuss a unique or recurrent origin for the mutation even when Bennani et al. (46) described an association with five RFLP haplotypes (i.e., I, III, V, IX and A) in Algeria, and Lemsaddek et al. (48), three RFLP haplotypes (IV, V and IX) in Morocco and Portugal.

Origin of Other β-Thalassemia Mutations in the Maghreb Countries

The codon 8 (–AA) is the second most common mutation encountered in Morocco. It appears rarely in Algeria and Tunisia. If this mutation was

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originally observed in a Turkish patient (90) and later in 5.5% of β-thalassemic Turkish alleles (91), nowhere is the frequency higher than in Russia (39%) and Azerbaijan (30%) (89). Some interesting features also appear concerning geographic distribution of the IVS-I-5 (G>T) and IVS-II-848 (C>A) mutations. They are restricted to central Algeria and northern and central Tunisia (33,46). It could reflect a common historical genetic event. The IVS-I-2 mutation, first detected in the region of Essouassi-El Djem in central Tunisia (76), accounts for 17% of β-thal mutations in central Tunisia, suggesting it is of Tunisian origin. Amazingly, some cases have recently been described in Morocco (48). Some sporadic genetic flow could explain this result. Hb Knossos [β27(B9)Ala→Ser, codon 27 (G>T)] has been observed both in Algeria (Algiers region) and Tunisia (92). A founder effect is probable through Palestine or Jordan where this mutation reaches a highest frequency (2.1 and 3.3%, respectively) (89). One case of the IVS-I-130 (G>A) β-thalassemic mutation is described in Morocco (Table 2). Such a mutant allele has also been described in a Turkish patient (93) and in an Egyptian patient (94). The −101 (C>T), β-thal allele is observed specifically in Morocco and eastern Algeria. It is sporadically found all around the Mediterranean Basin but with very low frequencies (around 1%). In this context, it seems very difficult to have some speculations concerning its origin. Finally, the sub-Saharan −29 mutation observed in Morocco (5.4%), in Algeria (3%) and sporadically in the northern part of Tunisia (1.08%), could be explained by caravan routes or could be the result of the Almoravid Dynasty that spread over a wide area of northwestern Africa (present-day Morocco, west part of Algeria, Mauritania, Senegal, Mali) and the Iberian peninsula (Southern Portugal and Spain) as suggested by Lemsaddek et al. (48).

Maghreb Autochthonous β-Thalassemia Mutations

A set of more than 10 mutations are clearly the result of autochthonous genetic occurrence. There are seven mutations prevalent in Morocco: −190 (G>A), −56 (G>C), Cap +20 (C>T) promoter mutation, codon 24 (T>A), IVS-II-726 (A>G), 25 bp deletion (+252 through +276) and the polyadenylation (poly A) site (AA*T*AAA>AA*C*AAA). Codon 30 (G>C) [Hb Kairouan, β30(B12)Arg→Thr], −87 (C>G), codons 25/26 (+T), and IVS-II-849 (A>C); these β-thal anomalies clearly seem to have a Tunisian origin. The codon 44 (−C), exclusively observed in Tunisia (2.4%), was first described in a Kurdish patient, but the highest frequency has been found in the Israeli population with a frequency of 9.4% (89). Four β-thal mutations could be considered autochthonous in Algeria: IVS-I-2 (T>A), IVS-II-843 (T>G), the −9 bp deletion/+31 bp insertion and poly A (AATAA*A*>AATAA*G*).

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Maghreb Autochthonous α-Thalassemia Mutations

Nine molecular defects responsible for α-thal and one α gene polymorphism have been reported in Tunisia (15,69) (Table 4). Six mutations were described in Algeria (70). The $-\alpha^{3.7}$ mutation is the most frequent allele in North Africa (15,69–71). It is specific to the Mediterranean regions but it reaches its highest frequencies in Iran (79.1%) (95) and Saudi Arabia (64%) (96) indicating that it could have been introduced in North Africa by Arab conquests. Previous studies have demonstrated that the majority of patients with the another two Mediterranean alleles [$- -^{\text{MED I}}$ and $\alpha^{\text{Hph I}}\alpha$ (IVS-I-2 (−5 nucleotide) (−5 nt) (α2) deletions], observed in both Tunisia and Algeria probably have a Sicilian (97) and a Western Mediterranean origin (98), respectively. On the other hand, the $\alpha^{\text{T–Saudi}}$ allele observed in Tunisia is more prevalent in the eastern part of the Mediterranean and in the Arabian Peninsula where it reaches 41% in Saudi Arabia (96). The $-\alpha^{4.2}$ mutation observed in Tunisia and Algeria seems to have a Southeast Asian origin. The Hb Groene Hart [α119(H2)Pro→Ser (α1)] mutation has been sporadically reported in association with the common $-\alpha^{3.7}$ deletion in heterozygotes of Moroccan (71) and Tunisian (68) origin. A Tunisian and then an autochthonous origin is plausible for some alleles [codon 23 (*G*AG>*T*AG) (α2), +6 (C>G) (α2) and the +14 (G>C) (α2) polymorphism in the 5′UTR (5′ untranslated region)] because until now they have only been observed in Tunisia (15,69). The $\alpha^{\text{Nco I}}\alpha$ (α2, codon 1, *A*TG>*G*TG) first described in an Italian patient and the $-(\alpha)^{20.5}$ of Mediterranean origin, were detected only in Algeria (70).

Maghreb Autochthonous Hemoglobin Variants

More than 20 Hb variants have been observed in North Africa (Table 3). The high variability is comparable with the range of variants described in the Mediterranean region. Moreover, many of these variants are common in different countries, denoting the ongoing genetic exchange occurring in this region over time. However, each country seems to have its own mutations that could have appeared de novo in its population. It is the case of Hb Tunis [β124(H2)Pro→Ser] (59), Hb Bab-Saadoun [β48(CD7)Leu→Pro] (61), Hb Tunis-Bizerte [α129(H12)Leu→Pro] (65), and Hb A_2-Pasteur-Tunis [δ59(E3)Lys→Asn] (45) for Tunisia, and Hb Setif [α94(G1)Asp→Tyr] (50), Hb Boumerdés [α37(C2)Pro→Arg] (55), Hb Loire [α88(F9)Ala→Ser] (56), Hb Melusine [α114(GH2)Pro→Ser] (64), Hb D-Ouled Rabah [β19(B1)Asn→Lys] (63) for Algeria, and Hb Kenitra [β69(E13)Gly→Arg] (54), Hb Dunn [α6(A4)Asp→Asn] (57), Hb Casablanca [β65(E9)Lys→Met; β122(GH5)Phe→Leu] (67) and Hb Newcastle [β92(F8)His→Pro] (48) for Morocco.

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Association Between Haplotype Polymorphism and the Moderate Phenotype of Sickle Cell Disease and β-Thalassemia

Some patients with sickle cell disease or β-thal show moderate phenotypes of their pathologies. Genetic polymorphisms have been described in association with these moderate phenotypes. Analysis using the RFLP haplotypes showed an association between haplotype IX and the IVS-I-2 (T>G) mutation for which homozygous patients show a moderate phenotype (35,76). The *Xmn*I polymorphism corresponding to the C>T variation at position −158 upstream to the Cap site of the $^{G}\gamma$ gene was found in strong association with the persistence of $^{G}\gamma/^{A}\gamma$ fetal rate in adulthood and most often a higher rate of Hb F in patients with sickle cell disease, β-thal and Hb E/β-thal (99). Some studies have shown that homozygosity for this polymorphism was significantly associated with an intermediate phenotype of β-thal in southeast Iran (100) and Turkey (101). In Tunisia, a recent study showed a strong association between the T allele of this polymorphism and the IVS-I-2 (T>G) mutation (unpublished data). In Algeria, this polymorphism was described long ago (87), and more recently in an Algerian patient showing a phenotype of β^0-thal intermedia (102). In Morocco, the *Xmn*I polymorphism has been shown in linkage with RFLP haplotypes III, IV and IX (48,80). Other studies have shown a positive correlation between this polymorphism and the mutation codon 8 (−AA) in linkage with haplotype IV (13). The determination of the molecular genetic marker for early childhood could help to provide better care for patients such as the identification of candidates for the pharmacological Hb F switch by hydroxyurea (13). However, other studies in Morocco showed an absence of correlation between the *Xmn*I polymorphism and Hb F levels (48,80). The (AT)xTy microsatellite polymorphism has also been described as influencing the phenotype of β-thal by increasing the rate of Hb F synthesis (103). On the other hand, some studies in Algeria have demonstrated the absence of this association (48,80) showing that correlation between the genotype and the phenotype can change from one population to another.

Finally, the genotype-phenotype relationship has recently been studied in a Tunisian family carrying the IVS-I-2 (T>G) mutation by studying the mRNA metabolism in the context of this molecular defect (104). The various splicing forms identified for these mRNA could provide new elements for understanding the moderate phenotype observed in patients carrying this mutation.

CONCLUSIONS

Hemoglobinopathies, particularly in the homozygous state, are severe diseases of the Mediterranean area. In North Africa, the prevalence of these

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diseases is more or less known. It is estimated at about 1.5 to 4.8% in the general population of the Maghreb countries. However, given the high rate of endogamy still observed in these countries, it is likely that genetic diseases in general and hemoglobinopathies in particular are especially prevalent. They now take an increasingly important place both in everyday medical practice and research. The development of genetics has led to a better understanding of the molecular basis of diseases studied, including the identification of mutations and polymorphisms associated with these mutations. It also makes possible an accurate diagnosis, including in the prenatal period, and the development of appropriate protocols for these diseases. Preimplantation diagnosis followed by IVF, represents "state of the art" procedures which allow the transfer of only unaffected embryos. This expensive technique is still not offered in clinical centers of the Maghreb countries. Finally, some hope could come from gene therapy. In the area of genetic disorders, ideas are changing the concept of preventive or curative medicine to that of predictive medicine.

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ACKNOWLEDGMENTS

The authors express their thanks to CNRS, the TEMPRA (Région Rhône-Alpes and Gouvernorat de Monastir), MIRA, CMCU, and OHLL (Origine de l'Homme, des Langages et des Langues) programs for supporting the training of Amel Haj Khelil in France. The authors wish to thank Dr. Faouzi Baklouti, (Research Director) INSERM, CNRS, UMR 5534 at the Center of Molecular and Cellular Genetics in Lyon, France; Amel Haj Khelil studied "Cell differentiation and alternative splicing" in his laboratory. We thank the referee for his very helpful comments.

Declaration of Interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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