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Identification of four common α-thalassemia gene deletions among a group with hemoglobinopathies in Sétif population, Algeria

Abstract: α-Thalassemia (α-thal) is one of the most common genetic disorders in the world. It is characterized by the absence or reduced expression of α-globin genes. This study was carried out to evaluate the allelic frequency of α-thal defects in a patient for the first time in Sétif (Algeria). One hundred and two patients with hemoglobinopathies from Sétif region, Algeria, presenting thalassemia were included in this study. Genomic DNA isolation was carried out according to standard methods. For identifying the α-thal genotype, investigation of α-globin gene deletions (-α3.7, -α4.2, -^{MED} and -α20.5) was performed by using multiplex-polymerase chain reaction (PCR). Among the three deletions found, the most mutations were the -α3.7 (10.78%), followed by the -^{MED} (5.88%) and -α20.5 (0.98%), whereas the -α4.2 deletion was not observed (0.0%). The allele frequency is 0.054 (11/204) for the 3.7 deletion, 0.029 (6/204) for the MED and 0.005 (1/204) for the 20.5. Molecular heterogeneity of mutations is typical of α-thal in Algeria. Our findings will be valuable and essential for the molecular diagnosis and prevention strategies of hemoglobinopathy gene mutations in the Algerian population.

Keywords: α-thalassemia; Algeria; frequency; mutation; Sétif.

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Introduction

Thalassemia (also called Mediterranean anemia) is a severe, usually fatal form of anemia. It comprises a group of genetic disorders of hemoglobin (Hb) synthesis caused by partial or total mutations that reduce or abolish the synthesis of α or β chains of globin in Hb. The two main types of thalassemia are called α and β, depending on which part of an oxygen-carrying protein in the red blood cells is lacking. Specifically, α-thalassemia (α-thal) is the most common Hb disorder in the world due to deletion or point mutations in α-globin genes [1]. It is characterized by the absence or reduction of α-globin synthesis in both fetal and adult life. A defect of α-chain synthesis results in an excess of γ- or β-globin chains and may lead to the formation of Hb Bart's (γ_4) or Hb H (β_4), respectively.

Of the numerous mutations that have been described, deletions at the α-globin gene locus account for the vast majority of α-thalassemia alleles [2]. Genomic deletions involving the α-globin gene cluster on chromosome 16p13.3 are the most frequent molecular causes of the disease.

Tetrahydrobiopterin is a cofactor required for nitric oxide (NO) production, and its synthesis rate-limiting enzyme is GTP cyclohydrolase (*GCH1*). A *GCH1* haplotype is defined by three linked single nucleotide polymorphisms correlated in the *MBL2* gene to vaso-occlusive events in children with sickle cell anemia [3]. The presence of α-thal may blunt the higher level of oxidative stress and the impaired bioavailability of NO observed in sickle cell trait [4]. Elevations of the pro-inflammatory cytokines TNF-α and IL-2 have been demonstrated in iron-overloaded patients with thalassemia [5]. An increased number of activated T cells and higher levels of serum neopterin were also observed in thalassemia patients, which suggest chronic stimulation of the immune system [6]. It was shown that the presence of α-thal co-morbidity has facilitated the development of anti-retroviral-induced anemia in human immunodeficiency virus infections [7].

Although α-thal is present throughout the world, its distribution varies greatly among different populations. The most widely occurring of these are the -$\alpha^{3.7}$ and -$\alpha^{4.2}$ single α-globin gene deletions, and the -^{MED} and -$(\alpha)^{20.5}$ double-gene deletions occur more frequently in the Mediterranean area. α-Thal is particularly prevalent in Southeast Asian, Middle Eastern and Mediterranean populations, including Algeria. We reported in Arab countries

an incidence of 1–58% for α-thal [8, 9]. α-Thal shows an incidence of 9.0% in Algeria [10].

The mutations of α-thal have been reported as $-\alpha^{3.7}$, $-^{MED}$, $-(\alpha)^{20.5}$ and $\alpha^{Hph1}\alpha$ in Algerian population [11, 12], and $-\alpha^{3.7}$ was determined as the most frequent haplotype [12].

In our previous study, in healthy individuals from the Sétif region, the prevalence of α-thal trait was found to be 6.5% [13]. Molecular characterization of α-thal defects in these subjects revealed that $-\alpha^{3.7}$ allele frequency was 3.3%. We have not found any other individual carrying the alpha 4.2 del, MED or 20.5 deletions in our study group [13].

Our present study investigated the frequency of α-thal gene mutations in patients from Sétif province located in the northeast of Algeria. These data are essential for establishing effective diagnostic guidelines and prevention strategies in this region.

Materials and methods

Patients

This research was carried out on 102 patients with hemoglobinopathies referred to the Setif Medical Biochemistry Laboratory. They originated mostly from the east of the country, around Sétif (northeastern Algeria). Informed consent was obtained from all the participants. The research protocol was approved by the Sétif Medical Faculty Ethics Committee. Blood samples for the study were transferred to Ankara (Turkey).

Sample collection and DNA extraction

Peripheral blood samples were collected by venipuncture, collected in test tubes that contained EDTA as an anticoagulant and maintained frozen at –20°C until the extraction of DNA and genotyping. DNA was extracted using the conventional phenol-chloroform method. After hemolysis of blood in hypotonic solution, DNA was isolated by a simple proteinase K treatment at 65°C in the presence of sodium dodecyl sulphate, followed by ammonium acetate precipitation of debris and ethanol precipitation of DNA. Then, DNA amount and DNA purity were quantified for each DNA sample by spectrophotometry (Nanodrop ND-1000) (Thermo Fisher Scientific, Waltham, MA, US). DNA samples were stored at –4°C until use.

Analysis of polymorphisms

Table 1 presents the nucleotide sequences of the primers used in the different polymerase chain reactions (PCRs). Genomic DNA was tested for the $-\alpha^{3.7}$, $-\alpha^{4.2}$, $-^{MED}$ and $-\alpha^{20.5}$ deletions using multiplex- PCR according to described methods. PCR A was a multiplex reaction containing the following primers: P51, P52, P54, P59 and P60. PCR B contained primers P55 and P54. PCR C was a multiplex reaction containing primers P71, P72 and P52. PCR D contained primers P55 and P52 (α-globin StripAssay, ViennaLab Diagnostics, Vienna, Austria).

Table 1 Nucleotide sequences of the primers used in the different PCR reactions.

Primer	Sequence
P51	5′-CTG CAC AGC TCC TAA GCC AC-3′
P52	5′-CCT CCA TTG TTG GCA CAT TCC-3′
P54	5′-CTC AAA GCA CTC TAG GGT CCA-3′
P55	5′-GTC CAC CCC TTC CTT CCT CA-3′
P59	5′-CTC TAG GTC ACC CTG TCA TCA-3′
P60	5′-CTC TGT CGT GTA GAC GCC GA-3′
P71	5′-TAC CCA TGT GGT GCC TCC ATG-3′
P72	5′-TGT CTG CCA CCC TCT TCTGAC-3′

Sources: Refs. [6, 7].

All PCRs were performed with Taq DNA polymerase (MBI Fermentas) (5 U/μL) using 50–100 ng of genomic DNA. Each 50-μL reaction contained 10× Taq buffer with $(NH_4)_2SO_4$ buffer, 25 mmol/L $MgCl_2$, 10 pmol/L per microlitre of primers, 2.5 μg/μL bovine serum albumin and 10% DMSO. Reactions were conducted in a thermal cycler (Biometra, Göttinng, Germany) with an initial 5-min denaturation at 94°C, followed by 35 cycles of 94°C denaturation for 1 min, 61°C annealing for 1 min and 72°C extension for 2 min. A final 10-min extension at 72°C completed the reaction. A total of 3.5 μL of each amplified product was analyzed by electrophoresis through a 2% agarose gel stained with ethidium bromide in 1× Tris-EDTA buffer at 80 V/cm for an hour.

Results

Table 2 presents the α-globin genotypes of 102 patients, and Table 3 presents the allele frequencies from Sétif region, Algeria. Among those patients tested for α-thal gene mutations, both α^+- and α^0- thal defects were found. Six different α-globin genotypes were identified: $-\alpha^{3.7}/\alpha\alpha$ (8.82%), $-\alpha^{3.7}/-\alpha^{3.7}$ (1.96%), $-^{MED}/\alpha\alpha$ (2.94%), $-^{MED}/-\alpha$ (0.98%), $-^{MED}/-\alpha^{3.7}$ (1.96%) and $-(\alpha)^{20.5}/\alpha\alpha$ (0.98) (Table 2).

Table 2 α-Thal genotype values in patients from Sétif region, Algeria.

Genotype	n	%
$\alpha\alpha/\alpha\alpha$	84	82.36
$-\alpha^{3.7}/\alpha\alpha$	9	08.82
$-\alpha^{3.7}/-\alpha^{3.7}$	2	01.96
$-^{MED}/\alpha\alpha$	3	02.94
$-^{MED}/-\alpha$	1	00.98
$-^{MED}/-\alpha^{3.7}$	2	01.96
$-(\alpha)^{20.5}/\alpha\alpha$	1	00.98
$-\alpha^{4.2}/\alpha\alpha$	0	00.00

Table 3 Allele frequency of α-thal in patients from Sétif region, Algeria (n=102).

Chromosomes		
Alleles	**n=204**	**Frequency, %**
αα	186	91.17
$-\alpha^{3.7}$	11	5.40
$-^{MED}$	6	2.94
$-(\alpha)^{20.5}$	1	0.49
$-\alpha^{4.2}$	0	0.00

The total α-thal frequency was 8.82%, with the $-\alpha^{3.7}$ haplotype being 5.40%; the $-^{MED}$ haplotype, 2.94%; the $-(\alpha)^{20.5}$ haplotype, 0.49%; and the-$\alpha^{4.2}$ haplotype, 0% (Table 3). Then, the most common α-thal genotypes in our patients from Sétif were heterozygous and homozygous $-\alpha^{3.7}$ single-gene deletions ($-\alpha^{3.7}/\alpha\alpha$, $-\alpha^{3.7}/-\alpha^{3.7}$). The second most frequent variant was $-^{MED}$ of mutated α-globin genes.

Discussion

α-Thalassemia (α-thal) is commonly seen in tropical and sub-tropical regions of the world, and carrier frequency can reach up to 80–90% in some regions [2]. The incidence of α-globin gene mutations among Arabs is well studied, and several investigators have determined the prevalence of these mutations in different populations. The lowest prevalence was reported in Iraq at 1% [8] and at <1% in Lebanon, Syria and Libya [14]. The highest frequencies were observed in Oman (58.3%) [15], Saudi Arabia (43.3%) [16] and UAE (49%) [9].

Algeria has a population of 38 million, approximately 1.5 million of whom live in Sétif. Sétif region is located in the high plateau of northeast Algeria, approximately 100 km from the Mediterranean Sea. We investigated the frequencies of α-globin mutations in patients from Sétif region, referred to our hospital.

Our study is the first to determine the frequency of α-globin gene polymorphisms in healthy patients from Sétif. Our values are intermediate between the highest and the lowest prevalence in Arab countries. However, the frequency of the mutant α-globin in our population was similar to that in Egypt (9.25%) [17], Tunisia (5.48%) [18] and Yemen (8.6%) [19].

Six mutations responsible for α-thal were described in Algeria [20]. The $-\alpha^{3.7}$ mutation is the most frequent allele in North Africa [21]. As expected, the most common mutation found was $-\alpha^{3.7}$, contributing 10.78% of mutated α-globin genes. This mutation was seen in 11 (5.4%) of 204 alleles. On the basis of our previous results, the prevalence of α-thal trait was found to be 6.5% in the healthy population from Sétif [13]. In a recent study, in addition to these mutations, $\alpha^{Nco\ 1}$ was shown and α-allele frequency was found to be 4.6% in randomly selected blood donors in Algiers (the capital city of Algeria), which is located at the Mediterranean Sea coast [20]. In the previous study, α-globin gene analysis showed the absence of the $-\alpha^{3.7}$ deletions [22]. Recently, Mesbah-Amroun et al. [20] reported that the carrier rate of the $-\alpha^{3.7}$ deletion was the most prevalent allele (2.9%), followed by the $-(\alpha)^{20.5}$ and $-^{MED}$ alleles (0.3% each). The reason for this difference in the two regions is unclear, given that there is no known contact, historic or economic, between these areas.

$\alpha^{3.7}$ Gene deletion has a worldwide distribution, and it is the most frequent mutation in many populations. It is specific to the Mediterranean regions, but it reaches its highest frequencies in West Asian countries such as Saudi Arabia (64%) [23], indicating that it could have been introduced to North Africa by Arab conquests [9].

Approximately 30–40% of African-American and African-Brazilian patients with sickle cell anemia are heterozygous, and up to3% are homozygous for the most common deletion-type α-thalassemia mutation $\alpha^{3.7}$ [24, 25]. The α-chains in thalassemic red blood cells can auto-oxidize, release heme and generate superoxide [26], which can then increase lipid peroxidation and thus enhance malondialdehyde levels [27].

The second most common α-thal deletion defect found in our cohort was $-^{MED}$ (5.88%) with an allele frequency of 2.94%, whereas the third most common was $-(\alpha)^{20.5}$ (0.98%). The $\alpha^{Nco\ 1}\alpha$, first described in an Italian patient, and the $-(\alpha)^{20.5}$, of Mediterranean origin, were detected only in Algeria [20] and are absent in neighboring Tunisia [28].

$-\alpha^{4.2}$ Gene deletion was not observed in our patients (0.0%). This is not in agreement with reports on Algerian α-thal patients from other studies [20]. The $-\alpha^{4.2}$ haplotype was not found in our patient, suggesting its low frequency in Algeria as in other Mediterranean populations [29]. The $-\alpha^{4.2}$ mutation observed in Algeria seems to have a South-east Asian origin [9].

Algiers is a large melting pot of local Afro-Mediterranean and Euro-Mediterranean invaders. The α-thal gene mutations clearly confirm this difference in the genetic structure of Algiers vs. Sétif gene pool on one hand and the Mediterranean coastal areas vs. the high plateau of northeast Algeria on the other hand. On one hand, this could be explained by the genetic contribution (gene flow) from some other Mediterranean areas, such as Italy, Greece or the Near East, and is perhaps related to the

allelic frequency cline, but, on the other hand, genetic drift operated in isolated and highly endogamous tribes from the mountains and plateaus.

To date, four alleles have been reported in the Algerian population, $-\alpha^{3.7}$, $-^{MED}$, $-(\alpha)^{20.5}$ and $-\alpha^{4.2}$, suggesting molecular heterogeneity in Algerians. The relatively small size of our population may not represent the total Algerian population. A study involving a larger segment of our population can clarify some of this heterogeneity. This reduced heterogeneity could be explained by the underestimation of these defects, which cannot be observed in adult life [9].

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