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α-globin gene deletion and point mutation analysis among Iranian patients with microcytic hypochromic anemia

We tested 67 Iranian individuals, presenting with low mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) levels, normal hemoglobin electrophoresis and iron status, for the presence of twelve common α-thalassemia gene deletions and point mutations. Five different mutations ($-\alpha^{3.7}$, $-\alpha^{4.2}$, $--^{MED}$, $-(\alpha)^{20.5}$, Hb Constant Spring) were identified in a total of 43 cases.

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α-thalassemia (α-thal) is one of the most common hemoglobin disorders in the world. It is characterized by the absence or reduced synthesis of α-globin chains. Unlike β-thalassemia (β-thal) mutations, which are mostly point mutations, the majority of α-thal mutations are deletions of one or both α-globin genes located on chromosome 16. The two most common single gene deletions are $-\alpha^{3.7}$ and $-\alpha^{4.2}$, while the Southeast Asian ($--^{SEA}$), the Mediterranean ($--^{MED}$), the Filipino ($--^{FIL}$) and the 20.5 kb ($-(\alpha)^{20.5}$) type are common α-thal double gene deletions. In addition, a small number of α-globin point mutations are known. Mutations are classified into α^+- and α^0-thalassemia to indicate, respectively, the reduced or absent output of α-globin chains from an affected chromosome. The clinical phenotype varies according to the number of affected genes.

Table 1. α-thal genotypes and associated hematologic values of 67 thalassemia patients.

Subject	*Mean Hb (g/dL)*	*Mean HbA_2 (%)*	*Mean MCV(fL)*	*Mutation*
23	14.1±1.0	2.4±0.6	73.9±6.4	$-\alpha^{3.7}/\alpha\alpha$
9	13.2±1.4	2.6±0.7	69.3±3.1	$-\alpha^{3.7}/-\alpha^{3.7}$
3	13.2±1.6	3.1±1.4	68.3±4.8	$--^{MED}/\alpha\alpha$
2	11.7±0.6	3.7±0.3	70.8±6.7	$-\alpha^{3.7}/-\alpha^{4.2}$
1	10.7	3.5	59.2	$-\alpha^{3.7}/--^{MED}$
1	13.6	3.8	66.0	$-\alpha^{4.2}/--^{MED}$
1	12.0	3.4	77.4	$-\alpha^{4.2}/\alpha\alpha$
1	11.4	2.4	68.3	$-(\alpha)^{20.5}/\alpha\alpha$
1	not available	3.4	76.5	$\alpha^{CS}\alpha/\alpha^{CS}\alpha$
1	14.8	3.5	78.4	$\alpha^{CS}\alpha/\alpha\alpha$
24	13.0±2.3	3.4±0.6	72.7±6.8	Normal

Carriers of three intact α-globin genes ($-\alpha/\alpha\alpha$) usually present with little, if any, detectable red blood cell abnormalities or globin chain imbalance, while double gene deletions ($--/\alpha\alpha$ or $-\alpha/-\alpha$) cause mild microcytic, hypochromic anemia with normal HbA_2 levels. Carriers with only one functional α-globin gene ($--/-\alpha$) present with hemoglobin (Hb) H disease, which is characterized by marked imbalance in globin chain synthesis ratios. Absence of all four α-globin genes (Hb Bart's hydrops fetalis syndrome) is incompatible with life.[1-3]

In an attempt to establish α-thal molecular diagnosis in Iran, a series of 67 individuals from different ethnic origins, randomly selected from a pool of patients presenting with low MCV (mean: 72.2±6.3 fL), low MCH (23.5±2.7 pg), normal or slightly reduced Hb (13.4±1.7 g/dL) levels, normal HbA_2 (3.0±0.8%), and negative results in β-thal genotyping (β-Globin StripAssay, Viennalab, Austria) were tested for the presence of α-thal mutations. Serum iron and ferritin levels, as well as total iron binding capacity were analyzed and confirmed to be in the normal range for all subjects in order to rule out iron deficiency. After comprehensive genetic counseling, blood was drawn from each individual, and genomic DNA was extracted according to established protocols.[4]

A polymerase chain reaction (PCR) method described by Baysal and Huisman[5] was used to detect the two α-thal single gene deletions $-\alpha^{3.7}$ and $-\alpha^{4.2}$. The Southeast Asian ($--^{SEA}$), Mediterranean ($--^{MED}$), Thai ($--^{THAI}$), Filipino ($--^{FIL}$) and 20.5 kb ($-(\alpha)^{20.5}$) double gene deletion variants, as well as point mutations Hb Constant Spring (cd 142: TAA-CAA), Hb Quong Sze (cd 125: CTG-CCG), Hb Pakse (cd 142: TAA-TAT), Hb Adana (cd 59: GGC-GAC) and cd 30 delGAG were investigated by a combined strategy based on multiplex PCR and reverse dot-blot analysis.[6,7] Southern-blotting using a ζ probe on DNA digested with BglII and Asp718[8] was performed to confirm the $-\alpha^{3.7}$,

$-\alpha^{4.2}$ and $--^{SEA}$ carrier status of patients.

Among the 67 Iranian subjects analyzed, α-globin gene mutations were identified in a total of 43 cases (64.2%). The most common α-thal genotypes were the heterozygous ($-\alpha^{3.7}/\alpha\alpha$) and the homozygous ($-\alpha^{3.7}/-\alpha^{3.7}$) forms of the common single gene deletion $-\alpha^{3.7}$, which were observed in 23 (34.3%) and 9 (13.4%) subjects, respectively. Furthermore, 3 subjects (4.5%) were $--^{MED}/\alpha\alpha$, and 2 subjects (3.0%) were $-\alpha^{3.7}/-\alpha^{4.2}$. One individual each (1.5%) was found positive for $-\alpha^{3.7}/--^{MED}$, $-\alpha^{4.2}/--^{MED}$, $\alpha^{4.2}/\alpha\alpha$, $-(\alpha)^{20.5}/\alpha\alpha$, $\alpha^{CS}\alpha/\alpha^{CS}\alpha$, and $\alpha^{CS}\alpha/\alpha\alpha$. In 24 individuals (35.8%) none of the twelve deletions or point mutations analyzed was detected (Table 1). Calculated allele frequencies among our cohort of patients were 32.8% for the $-\alpha^{3.7}$ deletion, 3.7% for the $--^{MED}$ deletion, 3.0% for the $-\alpha^{4.2}$ deletion, 2.2% for Hb Constant Spring (α^{CS}), and 0.7% for the $-(\alpha)^{20.5}$ deletion.

α-thal is not as prevalent as β-thal in Iran, but a significant number of cases with reduced MCV and MCH, normal Hb electrophoresis and normal iron status, are being referred to molecular biology clinics throughout the country as suspected cases of α-thal or normal HbA_2 β-thal. During earlier studies by our group, a high prevalence of the $-\alpha^{3.7}$ single gene deletion among such patients was noted.[10] In agreement with these preliminary data, our present study confirms that $-\alpha^{3.7}$ is by far the most common cause of microcytic, hypochromic anemia in Iran. In addition, we observed other α-globin deletions and point mutations in considerably lower frequencies. We will now use DNS sequencing to investigate the patients without known mutations for possibly abnormal hemoglobins.

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Incidence and characteristics of myelodysplastic syndromes in Ourense (Spain) between 1994-1998

The very few reference epidemiological studies on myelodysplastic syndromes (MDS) have been carried out in Europe: Germany, France, UK and Sweden. We present the first Spanish study on the incidence and characteristics of MDS. The incidence rates, distribution by FAB subtypes, sex and age groups are within the ranges established by the reference studies with minimal differences which we point out and attempt to explain.

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The province of Ourense is located in the southeast of Galicia, Spain. It has 346,913 inhabitants, according to the 1996 Renovation Census.[1] These inhabitants form a stable population with minimal migratory movements. Twenty-eight percent of the population are 65 years of age or older. Around 60% of the province's population lives in a rural environment.[2] There are no nuclear plants nor oil refineries in this region. The province's hospital assistance is distributed between three centers: the Complexo Hospitalario de Ourense (CHOU), the Hospital Comarcal de Valdeorras and the Fundación Hospital Verín, the only provincial centers in which diagnosis and follow-up of MDS patients are carried out. The source for identifying the cases was the bone marrow databases at these hospitals. The sample consists of all the patients diagnosed with MDS according to the FAB classification criteria[3] from January 1, 1994 up to December 31, 1998.

During the study period, 140 new cases of MDS were diagnosed. The crude incidence rate was 8.07/100,000/year (9.11 in men and 7.1 in women). The age standardized incidence rates were: 0 for age <50, 4.4 for age 50-59, 6.8 for age 60-69, 25.5 for age 70-79 and 56.7 for age >79. The incidence and the sex ratio increased with age; therefore, in the ≥ 70 year old age group, the incidence in men is 48.1/100,000/year, whereas in women it is 29.4/100,000/year (Table 1). The mean age at diagnosis was 77.5 years (CI 95%: 75.9, 79.0) with no significant differences between sexes (men 76.9 years and women 78.1 years, p=0.45) nor between FAB subtypes. Eighty-