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## ARTÍCULO ORIGINAL

# Molecular characterization of alpha-thalassemia in the Mexican population

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### ABSTRACT

**Background.**  $\alpha$ -Thalassemia ( $\alpha$ -Thal) has been poorly characterized at the molecular level in Mexico. **Methods.** 106 consecutive individuals identified in Laboratorios Clínicos de Puebla, with either hypochromia ( $MCH < 24 \mu g$ ) and/or microcytosis ( $MCV < 75 \text{ fl}$  in women or  $< 80 \text{ fl}$  in man), without iron deficiency, with or without anemia were investigated in this study, along a 16 month-period.  $\alpha$  and  $\beta$ -Thal were looked for, the former were characterized at the molecular level. **Results.** Out of the 106 consecutive cases with hypochromia and/or microcytosis and normal levels of protoporphyrin zinc complex, 48 cases (45.3%) had thalassemia (37 cases of  $\beta$ -Thal and 11 cases of  $\alpha$ -Thal), whereas in 58 cases (54.7%) a definite diagnosis could not be established. Of the  $\alpha$ -Thal cases, 8 were heterozygous and two were homozygous for the  $-\alpha^{3,7}$  deletion, whereas one case was heterozygous for the  $\alpha 2^{Hph}$  allele. **Conclusions.** Only few of the  $\alpha$ -Thal alleles tested were found, thus the  $\alpha$ -thalassemic mutations, present in the studied population, seem to be rather heterogeneous.

### Caracterización molecular de talasemia alfa en una población mexicana

### RESUMEN

**Antecedentes.** En México, la  $\alpha$ -talassemia ( $\alpha$ -Thal) ha sido pobremente caracterizada a nivel molecular. **Métodos.** Se estudiaron 106 individuos consecutivos identificados en los Laboratorios Clínicos de Puebla, con hipocromia ( $CMH < 24 \mu g$ ) y/o microcitosis ( $VCM < 75 \text{ fl}$  en mujeres o  $80 \text{ fl}$  en hombres), sin deficiencia de hierro, con o sin anemia, durante un periodo de 16 meses. Se investigaron  $\alpha$  y  $\beta$ -Thal; las primeras fueron caracterizadas a nivel molecular. **Resultados.** De los 106 casos consecutivos estudiados con hipocromia y/o microcitosis, y niveles normales del complejo de protoporfirina-cinc, 48 casos (45.3%) tenían talasemias (37 de ellos  $\beta$ -Thal y 11  $\alpha$ -Thal), mientras que en 58 casos (54.7%) no pudo establecerse un diagnóstico definitivo. De las talasemias  $\alpha$ , ocho casos eran heterocigotos y dos homocigotos para la delección  $-\alpha^{3,7}$ , mientras que sólo un caso resultó heterocigoto para el alelo  $\alpha 2^{Hph}$ . **Conclusiones.** De los alelos  $\alpha$ -Thal estudiados sólo se encontraron algunos, de lo que se infiere que en la población estudiada esas mutaciones parecen ser bastante heterogéneas.

**Key words.** Thalassemia. Anemia. Mexico.

### INTRODUCTION

The thalassemias result from deficient synthesis of one or more of the polypeptide chains of the normal human hemoglobin; this primary feature is a quantitative one and contrasts with the qualitative changes of hemoglobin structure that characterize the hemoglobinopathies.<sup>1</sup> Thalassemia is considered one of the most common genetic disorders worldwide: As far as  $\beta$ -Thal is concerned, about 3% of the world's population (180 mi-

llion people) carry  $\beta$ -Thal genes,<sup>2</sup> these genes being particularly prevalent in inhabitants of Italy and Greece, the highest prevalence of the carrier state being found in Sardinia (34%), the delta region of the Po river near Ferrara (20%) and Sicily (10%). The prevalence of the carrier state of  $\beta$ -Thal in Mexico is not low and there are data which suggest that the condition is not infrequent,<sup>3-5</sup> in addition, clusters of the condition in our country with a prevalence of up to 15% have been identified,<sup>6,7</sup> with data which suggest that  $\beta$ -Thal genes

in some places are autochthonous<sup>6</sup> and in others imported from the Mediterranean area.<sup>7</sup> Concerning  $\alpha$ -Thal in Mexico, the information is even scantier:  $\alpha$ -Thal has been found to be responsible for 1% of the hypochromic microcytic anemias in Mexico, this figure being about one half of that of  $\beta$ -Thal.<sup>4</sup> The thalassemic syndromes may result in red blood cell hypochromia and/or microcytosis with or without anemia, conditions that can mimic iron-deficiency states.<sup>1</sup> In previous studies,  $\alpha$ -Thal alleles ( $\alpha^{3.7}$ ,  $\alpha^{Hph}$ ,  $\alpha^{SEA}$ ,  $\alpha^{FIL}$ ) have only been identified in individuals.<sup>8,9</sup> We have analyzed the prevalence of both  $\alpha$  and  $\beta$ -Thal in a referral center of patients with hematological diseases. Propositi for  $\alpha$ -Thal were further characterized at the molecular level.

## MATERIAL AND METHODS

### Patients

The blood samples of 106 consecutive individuals identified in Laboratorios Clínicos de Puebla, with either hypochromia (MCH < 24 pg) or microcytosis (MCV < 75 fl in women or < 80 fl in man), without iron deficiency (PPZ < 80 mmol), with or without anemia were prospectively investigated in the study, along a 16 month-period. Written informed consent was obtained in all individuals.

### Quantification of Hemoglobin A<sub>2</sub> (HbA<sub>2</sub>)

In all samples, the quantification of HbA<sub>2</sub> was performed by ionic exchange column chromatography using the commercial Beta-Thal HbA<sub>2</sub> Quick Column Kit (Helena Laboratories) according to the provided instructions. Levels of HbA<sub>2</sub> above 3.8% were considered as indicative of  $\beta$ -Thal.

### DNA extraction

High molecular weight DNA was extracted from the samples remaining after exclusion of  $\beta$ -Thal according to standard protocols.<sup>10</sup>

### Detection of the $\alpha$ genes mutations

The genotype  $\alpha^{4.2}$  was determined using the primers described in Baysal and Huisman.<sup>11</sup> For the detection of the normal allele, 2  $\mu$ l of DNA (aprox. 1  $\mu$ g) in a final volume of 50  $\mu$ l were subjected after denaturation at 95 °C for one min to 35 cycles (15s 94 °C, 20s 65 °C, 20s 72 °C) and a final elongation step of 2 min at 72 °C. The amplification was performed as a hot-start PCR with AmpliTaq under the following

conditions: 2U AmpliTaq DNA polymerase (Perkin Elmer), 67 mM Tris/HCl pH 8.8, 16.6 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 2 mM MgCl<sub>2</sub>, 10 mM 2-ME, 8% DMSO, 0.1% gelatine, and 0.2 mM of each dNTP. For detection of the amplification products 8  $\mu$ l were separated on a 4.5% polyacrylamide gel. The  $\alpha^{4.2}$  allele was amplified with the Expand Long Template DNA Polymerase (Roche Molecular Systems) as a hot-start PCR with AmpliTaq using the buffer 3 supplied with the enzyme (50 mM Tris/HCl pH 9.2, 16 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 2.25 mM MgCl<sub>2</sub>, and 0.2 mM of each dNTP, temperature profile: 1 cycle: 1 min 95 °C; 10 cycles: 15s 95 °C, 50s 65 °C, 45s 68 °C, 20 cycles: 15s 95 °C, 50s 65 °C, 45s, with 20s increments at each cycle, 68 °C; 1 cycle: 2 min 68 °C). The detection of the genotype  $\alpha^{3.7}$  was performed as outlined by Bergstrom-Jones and Poon.<sup>12</sup> The samples homozygous for  $\alpha^{3.7}$  were confirmed with a 7-deletion multiplex PCR as described by Tan, *et al.*<sup>13</sup> Additional deletional  $\alpha$ -Thal ( $\alpha^{SEA}$ ,  $\alpha^{THAI}$ ,  $\alpha^{20.5}$ ,  $\alpha^{MED}$ ,  $\alpha^{FIL}$ ) included in this multiplex PCR were also searched for in the remaining samples as indicated. Non-deletional  $\alpha$ -Thal ( $\alpha^{2Hph}$ ,  $\alpha^{2Neo}$ ,  $\alpha^{TSaudi}$ ,  $\alpha^{Nco}$ ) were screened as described elsewhere.<sup>14</sup> The  $\alpha^{2Hph}$  mutation was confirmed by direct DNA sequencing with an automated sequencer (3.77ABI, Applied Biosystems, CA, USA), with primers previously described.<sup>15</sup>

## RESULTS

### Overall results

Out of the 106 consecutive cases with either red blood cell hypochromia or microcytosis, without iron deficiency, 48 cases (45.3%) had thalassemia (37 cases of  $\beta$ -Thal and 11 cases of  $\alpha$ -Thal), whereas in 58 cases (54.7%) a definitive diagnosis could not be established.

### $\alpha$ -thalassemia cases

In this subset of 11 individuals, there were two homozygotes ( $\alpha^{3.7}/\alpha^{3.7}$ ) and 9 heterozygotes (8  $\alpha^{3.7}/\alpha\alpha$ ; 1  $\alpha^{Hph}\alpha/\alpha\alpha$ ). Interestingly, six patients had normal levels of hemoglobin despite the thalassemia.

### Undefined cases

In this group of cases, several possibilities can be considered: There are either other types of thalassemias which were not assessed in this study, or patients with other conditions different from iron deficiency or thalassemia leading into either red blood cell microcytosis or hypochromia.

## DISCUSSION

The most frequent cause of anemia as the primary complain in the studied population is iron deficiency,<sup>4</sup> which represents 69.6% of patients;<sup>16</sup> on the other hand, β-Thal represents 6% of all patients studied and treated at this institution.<sup>16</sup>

In this large prospective study iron deficiency anemia represented 78.8% of all cases of microcytosis and/or hypochromia, a figure similar to that previously reported<sup>16</sup> in which iron deficiency was found to be responsible for the 69% of patients with hypochromic microcytic anemia. In the present study we have investigated individuals with or without anemia, this criterion probably accounting for the observed differences. We have also found that the thalassemic syndromes account for approximately 10% of cases with red blood cells microcytosis and/or hypochromia and that β-Thal was at least three times more frequent than α-Thal in this group of Mexican mestizos. It is important to mention that other types of α-Thal different from the ones that we have searched for in this paper may account for some additional cases of thalassemia and may also be included in the subset of individuals in which a definite diagnosis could not be established; accordingly, it is possible that we may be underestimating the prevalence of α-Thal in this study. Interestingly, this study does not identify additional alleles to those previously described in the Mexican mestizos,<sup>8,9</sup> thus we confirm that the α-thalassemic mutations, present in the population studied, seem to be rather heterogeneous.<sup>9</sup>

In a study informed from the United States, there are data showing that the most frequent cause of anemias in the general practice, the "common anemias" is thalassemia, since β- added to α-Thal are more frequent than iron deficiency anemias.<sup>17</sup>

In summary, we have found that β and α-Thal are not infrequent in the population studied: they are both less frequent than iron deficiency, but together more prevalent than megaloblastic erythropoiesis as a cause of anemia. Accordingly, the thalassemias should not be considered as exceptional in Mexico, and individuals with red blood cell microcytosis and/or hypochromia, with or without anemia should be screened for thalassemia, prior to labeling them as iron-deficient and offering them, sometimes erroneously, iron supplements.<sup>4,17</sup>

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*Recibido el 27 de junio de 2005.*

*Aceptado el 17 de enero de 2006.*