

## Original Article

# A Novel Alpha-thalassemia Nonsense Mutation in HBA2: C.382 A > T globin Gene

Mohammad Hamid PhD<sup>1,2</sup>, Hanieh Bokharaei Mercı MSc<sup>1</sup>, Hamid Galehdari PhD<sup>3</sup>, Ali Hossein Saberi PhD<sup>2,3</sup>, Bijan Kaikhaei MD<sup>4</sup>, Marziye Mohammadi-Anaei BSc<sup>2</sup>, Ahmad Ahmadzadeh MD<sup>5</sup>, Gholamreza Shariati PhD<sup>2,3</sup>

## Abstract

In this study, a new alpha globin gene mutation on the  $\alpha_2$ -globin gene is reported. This mutation resulted in a Lys > stop codon substitution at position 127 which was detected in four individuals (three males and one female). DNA sequencing revealed this mutation in unrelated persons in Khuzestan province, Southwestern Iran of Lor ethnicity. This mutation caused no severe hematological abnormalities in the carriers. From the nature of substituted residues in  $\alpha_2$ -globin, it is widely expected that this mutation leads to unstable and truncated protein and should be detected in couples at risk for  $\alpha$ -thalassemia.

**Keywords:** Iran,  $\alpha$ -globin gene mutation,  $\alpha$ -thalassemia

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## Introduction

Alpha thalassemia, the most common inherited disorder of hemoglobin in the world, is caused by deletion and/or mutation of  $\geq 1$  of the four alpha-globin genes.<sup>1,2</sup> The Iranian population is composed of a mixture of different ethnic groups and the most frequent lesion reported so far is the  $-\alpha^{3.7}$  mutation.<sup>2</sup> Khuzestan province located in southwestern Iran comprises different ethnicities including Arab, Lor and Persian. The  $-\alpha^{3.7}$  single gene deletion was the most frequently identified variant, representing 62.6% of the total in Khuzestan province.<sup>3</sup> In the present study, we describe heterozygosity for a new nonsense mutation on the  $\alpha_2$  globin gene.

## Materials and Methods

This study included individuals referred to the Narges Prenatal Diagnostics and Medical Genetics Laboratory as part of a national program for prevention of thalassemia. The red blood cell indices and hemoglobin analysis were carried out according to standard methods. After obtaining written informed consent, molecular studies were conducted on genomic DNA isolated from peripheral blood cells by salting-out procedure.<sup>4</sup> For identifying  $\alpha$ -thalassemia genotype, investigation of common Mediterranean  $\alpha$ -globin gene deletions ( $-\alpha^{3.7}$ ,  $-\alpha^{4.2}$ ,  $-\alpha^{20.5}$  and  $-\alpha^{MED}$ ) was performed by Gap-PCR as described previously;<sup>5</sup> the entire  $\alpha$  and  $\beta$ -globin genes were amplified and DNA sequenced, ABI -3130 (Applied Biosystems, Foster City, CA, USA).

**Authors' affiliations:** <sup>1</sup>Department of Molecular Medicine, Biotechnology Research Center, Pasteur Institute of Iran, Tehran, Iran, <sup>2</sup>Narges Medical Genetics & PND Laboratory, Kianpars, Ahvaz, Iran, <sup>3</sup>Department of Medical Genetic, Faculty of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran, <sup>4</sup>Research Centre of Thalassemia and Hemoglobinopathies, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran. <sup>5</sup>Health Research Institute, Research Centre of Thalassemia and Hemoglobinopathies, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

**Corresponding author and reprints:** Gholamreza Shariati PhD, Narges Medical Genetics & PND Laboratory, No. 18, East Mihan Ave, Kianpars, Ahvaz, Iran. Tel: +98 6113336681, Fax: +98 6113336682, E-mail: Shariatig@yahoo.com.

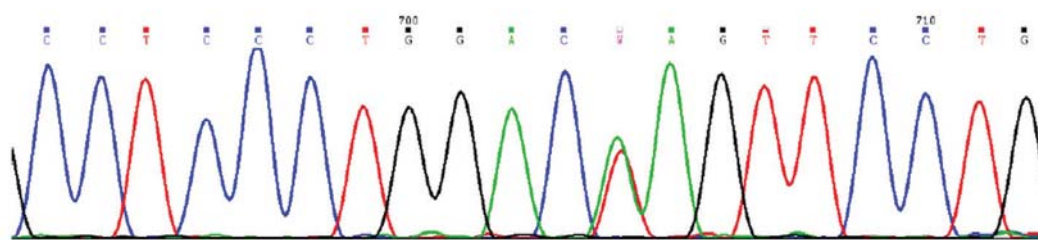
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## Results

Hemoglobin (Hb) analysis by applying cellulose acetate electrophoresis at alkaline pH (8.4 – 8.6) did not indicate any abnormal Hb fraction. Sequencing of the  $\alpha$ -globin gene of subjects detected a novel mutation  $\alpha_2$  cd127 which results in premature stop codon in the third exon of  $\alpha_2$ -globin genes (Figure 1). The mutation  $\alpha$ pha2 [ $\alpha$ 127 (H10) Lys > stop] was detected in four individuals (three males and one female) showing a phenotype of moderate anemia, mild microcytosis and moderate hypochromia. Subjects were unrelated of Lor ethnicity from Khuzestan province. The hematological and molecular features of this mutation in carrier individuals are summarized in Table 1.

## Discussion

The A > T mutation at codon 127 of the  $\alpha_2$ -globin genes leads to a substitution of Lysine by a stop codon which in heterozygosity shows no detrimental impact in carriers. Nonsense mutations occur when a premature stop codon is introduced in the DNA sequence, resulting in a shorter and unfinished protein product. The truncated protein is not able to form a stable dimer and is degraded by the proteolytic pathway.<sup>6</sup> However, we can also expect this mutation to result in synthesis of unstable truncated proteins or an unstable RNA transcript. The Lor population of Khuzestan province is characterized by marriage at a young age, large family sizes and customary consanguineous marriages. Moreover, in this area, as a previous report has shown the high frequency of  $\alpha_1$  and  $\alpha_2$ -globin mutations,<sup>3</sup> it is expected that novel mutations will be identified with high frequency.<sup>3</sup> In conclusion,  $\alpha_2$  cd127 are new mutations which cause no severe hematological abnormalities among these carriers, but they lead to unstable and truncated protein or an unstable RNA transcript. Hence, we recommend the screening of non-deletional mutations after the most common deletions were discarded and this mutation should be detected in couples at risk for  $\alpha$ -thalassemia.



**Figure 1.** Sequencing of the  $\alpha$ -globin gene

**Table 1.** The hematological parameters for  $\alpha$ -thalassemia genotype in carrier individuals

Subject	Three male persons	One female person
Hb (g/dl)	14.56 $\pm$ 0.92	13.5
RBC ( $10^{12}/L$ )	6.03 $\pm$ 0.31	5.9
MCV (fl)	73.36 $\pm$ 1.96	74
MCH (pg)	24.0 $\pm$ 0.36	22.9
Hb A2 (%)	2.4 $\pm$ 0.5	1.9
$\beta$ -genotype	N/N	N/N
$\alpha$ -genotype	$\alpha^{CD 127(AAG>TAG)} \alpha/\alpha\alpha$	$\alpha^{CD 127(AAG>TAG)} \alpha/\alpha\alpha$

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