

## Case Report

# Identification of IVS-I (-1) (G > C) or Hb Monroe as a Report on the Beta-globin Gene with a Beta-thalassemia Minor Phenotype in South of Iran

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## Abstract

We described the first report of IVS-I (-1), codon 30 (G > C) or Hb Monroe in five individuals from four unrelated families in Khuzestan Province. Polymerase chain reaction (PCR) followed by sequencing of the beta-globin gene confirmed the presence of Hb Monroe in the heterozygous form which causes beta-thalassemia due to missplicing in the course of mRNA processing. This mutation has been described in individuals originated from Arabic and Behbahani origins, Ahvaz City, south of Iran. The knowledge of the beta-globin variants present in the Iranian population is essential for the molecular diagnosis and prevention of hemoglobinopathies.

**Keywords:** Beta-globin gene, Iran, thalassemia (thal)

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## Introduction

Beta-thalassemia ( $\beta$ -thal) syndrome is one of the most frequent hereditary diseases in the Mediterranean region, comprising about 280 mutations.<sup>1</sup> These mutations lead to reduction ( $\beta^+$  type) or absence ( $\beta^0$  type) of  $\beta$ -globin chain production, resulting in  $\beta$ -thal phenotype. The majority of  $\beta$ -thal mutations are point mutations and rarely are microinsertion/deletion.<sup>2</sup> Hb Monroe results from a splice site point mutation (G > C) in the last nucleotide of  $\beta$ - globin exon 1, which is also the penultimate nucleotide of codon 30 of the  $\beta$ - globin peptide (AGG- > ACG; Arg- > Thr).<sup>3</sup> This mutation as  $\beta^0$  type mutation prevents splicing completely and no normal mRNA is formed. We here describe the first report of IVS-I (-1), codon 30 (G > C) or Hb Monroe in five individuals from four unrelated families in Khuzestan Province.

## Materials and Methods

This study included individuals referred to the Narges Prenatal Diagnostics and Medical Genetics Laboratory during the one year for carrier detection as part of a national program for the prevention of thalassemia.

Analysis of red blood cell indices and Hb analysis were carried out according to standard methods. Following obtaining written informed consent, molecular studies were conducted on genomic DNA isolated from peripheral blood cells by a salting-out procedure. For identifying  $\alpha$ -thal genotype, investigation of common

Mediterranean  $\alpha$ -globin gene deletions was performed by Gap-PCR as described elsewhere.<sup>4</sup> Beta- globin genes were amplified and directly sequenced by the chain termination method on an ABI-3130 (Applied Biosystems, Foster City, CA, USA).

## Results

In this study, we described the first report of IVS-I (-1), codon 30 (G > C) or Hb Monroe in five individuals from four unrelated families in Khuzestan Province. They were referred to the Laboratory for carrier detection as part of a national program for the prevention of thalassemia with mild anemia, microcytosis, and hypochromia. The hematologic and molecular data of the study subjects are summarized in Table 1. Hb X has not been found in these cases by a cellulose acetate electrophoresis, so it is an unstable hemoglobin variant. Sequencing chromatograms of this mutation is shown in Figure 1.

## Discussion

Hb Monroe was first found in a transfusion-dependent 15-year-old black girl from the USA with the promoter mutation A > G at position -29.<sup>5</sup> This mutation was also reported from other populations, such as Indians,<sup>6</sup> Tunisians,<sup>7</sup> and Tajiks.<sup>8</sup> This is the first report of this mutation in south of Iran and second reported in Iranian population. It was reported in a patient from Qazvin Province who inherited this mutation with IVSII-1.<sup>9</sup> This missense mutation not only leads to undetectable mutant peptide and transcript but also interferes with the expression of wild type allele.<sup>3</sup> Therefore, it plays a role in pathogenesis by reducing the splicing efficiency.

In conclusion, Iran as a country with multiethnic groups has different provinces with their own characteristic spectrum of beta-globin gene mutations. Searching for and identifying rare or new mutations is a constant priority in population screening, genetic counseling, and prenatal diagnosis of thalassemia.

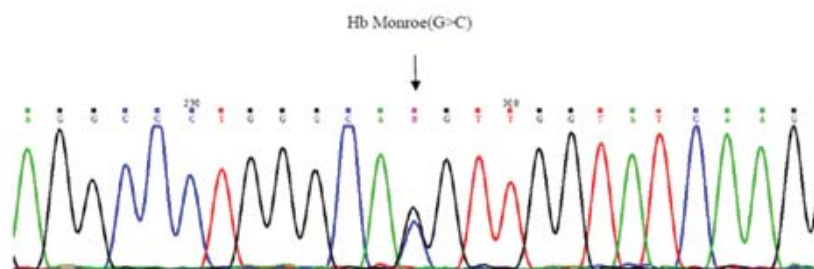
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**Table 1.** The hematologic and molecular data

Subject	G.Ka	B.Ka	N.Ni	L.Se	A.Se
Age (year)	25	27	22	21	30
Sex	F	M	F	F	M
Hb (g/dL)	11.0	12.3	12.0	8.7	11.0
RBC (10 <sup>12</sup> /L)	5.65	6.71	6.09	4.58	5.91
MCV (fL)	64.4	62.6	64	60.2	68.1
MCH (pg)	19.5	18.3	19.7	19	19.8
MCHC (%)	----	----	30.7	31.5	----
Hb A (%)	93.8	----	92.9	93.9	95.1
Hb F (%)	2.6	----	1	0.8	0.6
Hb A2 (%)	3.6	----	6.1	5.3	4.3
$\beta$ -genotype	Hb Monroe/N	Hb Monroe/N	Hb Monroe/N	Hb Monroe/N	Hb Monroe/N
$\alpha$ -genotype	$\alpha\alpha/\alpha\alpha$	$\alpha\alpha/\alpha\alpha$	$\alpha^{-3.7}\alpha/\alpha\alpha$	$\alpha\alpha/\alpha\alpha$	$\alpha\alpha/\alpha\alpha$
Origin	Behbahani	Behbahani	Arab	Arab	Arab

**Figure 1.** Sequencing chromatograms of Hb Monroe (G > C) mutation.

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