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# A SILENT COMPOSITE HEMOGLOBINOPATHY CHARACTERIZED BY GENE SEQUENCING

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## RESUME

*Nous rapportons le cas d'une patiente Tunisienne âgée de 35 ans présentant une anémie chronique non explorée depuis longtemps. Les investigations de laboratoire utilisant le séquençage de l'ADN mettent en évidence une double hétérozygotie Hb O Arab/cd 39  $\beta^0$ -thalassémie. C'est la première fois qu'un tel génotype a été exploré par séquençage du gène.*

**Mots clés:** Anémie chronique, association Hb O-Arab/ $\beta^0$ -thalassémie, séquençage d'ADN.

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## ABSTRACT

*We report the case of 35 years old Tunisian women with a chronic anemia non investigated for a long time. Laboratory analysis using advanced technology of DNA sequencing revealed a compound heterozygote for Hb O Arab and cd 39  $\beta^0$ -thalassemia. It's the first time that such a genotype has been characterized by gene sequencing.*

**Key words:** Chronic anemia, Hb O-Arab/ $\beta^0$ -thalassemia association, DNA sequencing.

## INTRODUCTION

HbO-Arab is an abnormal  $\beta$  variant, characterized by the substitution of a glutamic acid by a lysine at position 121 of the beta chain ( $\beta 121$ : Lys $\rightarrow$ Glu)<sup>1</sup>. The electrophoretic mobility of Hb OArab is similar to that of Hb C, Hb E, and Hb A2 on cellulose acetate at alkaline pH. In contrast, Hb OArab migrates close to Hb S on citrate agar at acidic pH<sup>2</sup>. Hb OArab trait have been reported in Saudi Arabia<sup>3</sup>, North Africa<sup>4</sup>, Sudan<sup>5, 6, 7</sup>, Yugoslavia<sup>8</sup>, Bulgaria<sup>9</sup>, Jamaica<sup>10</sup>, the Mediterranean area<sup>11</sup>, and the United States<sup>12</sup>. Few cases have been described among Tunisian and were characterized only by protein procedure<sup>13, 14</sup>. It is an abnormal Hb variant without significant

clinical consequences either at the heterozygous or homozygous state. However, when it is associated with sickle cell disease, severe anemia could be observed leading to major syndromes<sup>15</sup>.

On the other side, when this variant is co-inherited with  $\beta$ -thalassemia, a discreet chronic microcytic hypochromic anemia is usually observed<sup>16</sup>. Consequently, patients with such a genotype might remain free of syndrome until another pathology requiring laboratory investigation occurred.

Here in, we report the case of a long time ignored doubly heterozygous HbO-Arab/ $\beta^0$ -Thalassemia discovered fortuitously upon a routine investigation for chronic microcytic anemia.

## MATERIAL AND METHODS

### Case presentation

The proposita is a 35 years old Tunisian women originating from a rural area of the North-Western part of the country where hemoglobinopathies are endemic<sup>17</sup>. The first examination at the admittance finds an anemic patient. The anamnesis did not reveal notable personal or family antecedents. In spite of this medical history, the patient was strongly suspected to present an abnormal hemoglobin syndrome since she was anemic and originated from an endemic area for hemoglobinopathies.

### Routine investigations

Blood samples were collected in EDTA as anticoagulant. Complete blood count was obtained using automatic cell counter (coulter counter ABX Mcro-60 OTR. Abx Diagnostics, Montpellier, France). Cell lysates were analysed by various electrophoretic systems including cellulose acetate at alkaline pH (8.6), agar citrate at acid pH (6.2) and isoelectric focusing (IEF) in poly-acrylamide gel using a mixture of ampholines, pH 5-8 and 7-9. Moreover, a further hemoglobin analysis and quantification were carried out by cation exchange HPLC using the D-10 system (Bio-Rad Laboratories) according to the procedure recommended by the manufacturer<sup>18</sup>.

### DNA analysis

DNA was obtained from peripheral blood leucocytes by the standard phenol/chloroform method. The three exons of the  $\beta$  globin gene were specifically amplified by conventional PCR as described previously<sup>19</sup>. Obtained amplicons were then purified and sequenced using the dye terminator method on an automatic sequencer (ABI PRISM™ 3130 DNA Genetic Analyzer; Applied BioSystems, Foster City, CA, the USA).

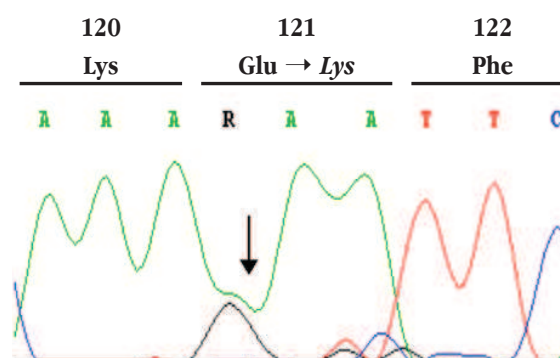
## RESULTS

### Laboratory investigations

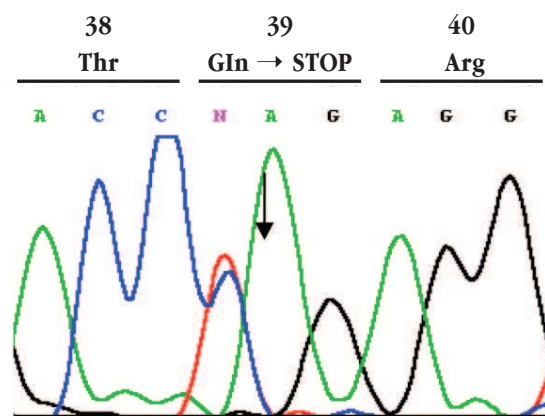
Hematological data highlighted a microcytic hypochromic anemia (Hb: 6.8 g/dL, MCV: 64.7 fL, MCHC: 31.1 g/dL, MCH: 20.1 pg) in spite of an elevated ferritinemia (365 ng/ml). Hb pattern obtained by HPLC and IEF showed a major fraction of Hb O Arab (89.7%), increased levels of Hb F and A<sub>2</sub> (respectively 6.2 and 4.1%). This profile suggested either homozygote Hb O-Arab or doubly heterozygote Hb O-Arab/ $\beta^0$ -thalassemia, since we

are in presence of a microcytic anemia with elevated Hb A<sub>2</sub> level.

In front of this situation and in the absence of family study (death of the two parents) a molecular investigation was undertaken allowing to obtain a final diagnosis of a doubly heterozygote O-Arab/ $\beta^0$  thal. Indeed, the  $\beta$ -globin gene sequencing showed two heterozygote mutations: a structural mutation at codon 121 (GAA>AAA) characteristic of the Hb O-Arab and a stop codon at position 39 CAG > TAG leading to  $\beta^0$ -thalassemia (Figure 1).



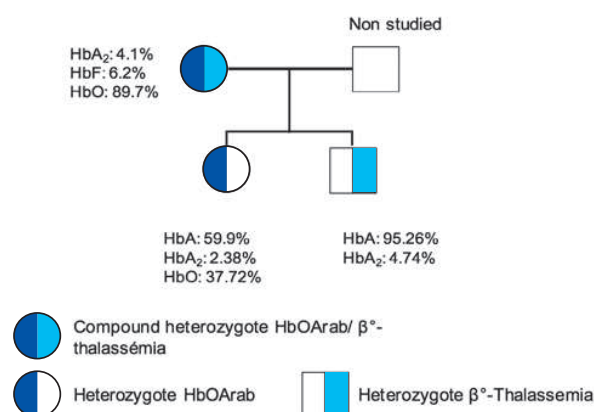
**A: heterozygote HbO-Ara  $\beta$ 121**  
GAA → AAA Glu → Lys



**B: heterozygote  $\beta$ 39**  
CAG → TAG Gln → STOP

**Figure 1** : Fluorescent- based automated DNA sequence analysis of the  $\beta$ -globin gene: **A** : Hb O-Arab, A>G substitution at codon 121; **B**: Abnormal sequence C >T: at codon 39.

These two alleles were later on observed in two of her descent, one of them was heterozygous  $\beta^0$ -thal (cd 39 CAG > TAG) the other was heterozygote HbO-Arab (Figure 2).



**Figure 2 :** Pedigree of the family showing the segregation of HbO and  $\beta$ -thalassemia in the offspring of the *proposita*.

## DISCUSSION

The first case of hemoglobin O-Arab was described in 1960 in an Arab child suffering from sickle cell disease syndrome<sup>20</sup>. The genetic alteration leading to this Hb was revealed initially by protein sequencing on 1962<sup>21</sup>. In Tunisia, although several hemoglobinopathies were described with high incidence, HbO-Arab remains until now as one of rare variants detected so far. Its incidence doesn't exceed 0.11 % and the most cases were doubly heterozygote of HbO with other hemoglobin abnormalities. They are mostly reported in fortuitous associations with other pathology<sup>22</sup>.

To our knowledge the majority of hemoglobin O Arab met in our country was described in association with other hemoglobin abnormalities during systematic surveys in regions at risk<sup>23</sup>. The most severe forms (association with sickle cell disease or thalassemia) remain under diagnosed because of the iron deficiencies interferences relatively dominant in rural environment. In some cases, HbO-Arab was discovered with different forms on biliary lithiasis and occasionally with hydatidosis<sup>24</sup>.

The patient which we present here had a chronic microcytic anemia previously considered as a common refractory iron deficiency. Hemoglobinopathy was suspected only at old age. It's the first time that a double heterozygote Hb O-Arab/ $\beta^0$ -thalassemia remains undiagnosed for a long time. This diagnosis delay could be explained by the fact that HbO/ $\beta^0$ -Thalassemia behaves as minor hemoglobin syndrome. However, previous clinical reported data on Tunisian HbO-Arab beta thalassemia indicate that some of them presented episodes of severe hemolytic anemia requiring blood transfusions in childhood<sup>25</sup>.

Finally, it is of great importance to notify that biological and molecular investigation might be performed when we face anemia especially in endemic region for hemoglobinopathies. Moreover, it would be judicious to explore hemoglobinopathy in order to improve the assumption of responsibility and to give the patient a complete and final diagnosis for a better prevention as well as an adapted treatment.

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