Fortuitous description of hemoglobin Hope in a high-level Tunisian athlete: molecular diagnosis and origin

Détection fortuite de l’hémoglobine Hope chez un athlète tunisien de haut niveau : diagnostic moléculaire et origine

Abstract. In this study we report the fortuitous description of hemoglobin (Hb) Hope in a Tunisian athlete. This Hb is one of hemoglobin variants that show a lower stability and oxygen affinity that is beneficial to tissue oxygen delivery. Hb Hope was isolated by automated high performance liquid chromatography and was unequivocally found to be Hb Hope using DNA-based methods: polymerase chain reaction, denaturing gradient gel electrophoresis, direct DNA sequencing. Restriction haplotype showed that this Hb was supported by the Mediterranean haplotype I. Hb Hope was identified at first in a black African-American family and later in several other black and non black ethnic groups. All these descriptions raise the question of the Hb Hope origin. Recently, Hb Hope was reported in Thai in association with the same Mediterranean haplotype I. This favors that Tunisian and Thai Hb Hope would share a common Mediterranean origin, thus suggesting the possibility of a Mediterranean gene flow. On another hand, the observation of Hb Hope in a high level athlete would suggest a selection pressure of this Hb variant due to higher physical aptitude.

Key words: haemoglobin, hemoglobinopathies, molecular diagnosis

Résumé. Dans cette étude, nous rapportons la description fortuite de l’hémoglobine (Hb) Hope chez un athlète tunisien de haut niveau. Cette hémoglobine est l’un des variants de l’hémoglobine qui montre une faible affinité pour l’oxygène qui serait bénéfique à sa distribution dans les tissus. Ce variant a été coélué avec l’hémoglobine A1c par chromatographie liquide à haute performance. Son identification en tant qu’Hb Hope a été rendue possible par les techniques de biologie moléculaire : la réaction de polymérisation en chaîne (PCR), l’électrophorèse sur gel en gradient dénaturant et le séquençage direct de l’ADN. L’étude des haplotypes de restriction par technique de PCR-RFLP (polymorphisme de longueur des fragments de restriction) a montré que cette Hb est supportée par l’haplotype méditerranéen de type I. L’Hb Hope a été identifiée d’abord dans une famille afro-américaine et plus tard dans plusieurs groupes ethniques. Toutes ces descriptions soulèvent la question de l’origine de l’Hb Hope. Récemment, l’Hb Hope a été rapportée en Thaïlande en association avec l’haplotype I. Cela suppose une origine méditerranéenne commune des allèles thaïlandais et tunisien. D’un autre côté, l’observation de l’Hb Hope chez un athlète de haut niveau pourrait suggérer une pression de sélection de ce variant en faveur d’une meilleure aptitude physique.

Mots clés : hémoglobine, hémoglobinopathies, diagnostic moléculaire
To date, close to 900 variants have been discovered and the count continues to rise [1]. The majority of them are beta chain variants. However, only a few have altered oxygenation properties and are clinically significant. The most worldwide common one is the hemoglobin S [β6(A6) Glu→Val (GAG→GTG)] that is responsible of sickle cell disease; this hemoglobin variant presents a high affinity for oxygen that contributes to its clinical severity. On the other hand, Hb Hope [β136(H14) Gly→Asp (GGT→GAT)], is one of hemoglobin variants showing a lower oxygen affinity thus explaining its usual innocuity. Initially described in a black family, then in other black and non-black families, Hb Hope raises three main questions, i) its diagnosis, as a rare unusual variant, ii) its origin since there are descriptions from African and non-African individuals, and iii) the selective pressures that would facilitate the survival and prevalence of this mutation [2].

Material and methods

Unexpectedly, the detection was done in a high-level (HL) athlete during a national haemoglobinopathy screening survey of athletes in the country. The subject was a 29-year-old female of non-Black African origin. She was engaged in the national Tunisian rugby team after 12 years of athletic competitions. She won two national championships in tracks and fields events (400-m and 4×400-m relay), and her records on the 200-m, 400-m and 800-m races were 26.4 s, 59.7 s and 2 min 17 s, respectively. Informed consent was obtained from the athlete and her parents. Blood samples were collected on EDTA at the National Centre of Sports Medicine and Sciences of Tunis. Haematological analyses were performed with an automated cell counter (ABX Micro-60-OT Abx® Diagnostics, Montpellier, France). Abnormal hemoglobin was characterized by automated cation exchange high performance liquid chromatography (HPLC) on Variant II (Variant II® - Bio-Rad Laboratories, Hercules, CA, USA) using Variant II™ HbA2/HbA1c Dual Program. DNA was isolated from peripheral leucocytes according to standard protocols [3]. To locate the exon presenting the nucleotide mutation, we explored the β-globin gene by denaturing gradient gel electrophoresis (DGGE) as previously described [4]; three fragments of the gene were analyzed: the first fragment consists of the promoter, the exon 1, the intron 1 and the 5’ part of exon 2. The second region comprises the 3’ part of the first intron, the second exon and the 5’ part of intron 2. The last fragment covers the 5’ extremity of the intron 2, the third exon and the 3’ untranslated terminal region. The fragment showing an atypical pattern at DGGE was sequenced using a Big-Dye-Terminator cycle sequencing Ready Reaction Kit and analyzed on an automated sequencer ABI prism 310 Genetic Analyzer (Applied Biosystems, Foster city, CA, USA). β-globin haplotype was established by mapping restriction fragment length polymorphisms (RFLP) using PCR based methods as described [5, 6]. The polymorphic pattern of the following seven restriction sites - Hind III 5’ to the e gene; Hinc II 3’ to the e gene; Hind III in the IVS2 of Gy and Ay genes; Hinc II 5’ and 3’ in the ψβ gene; Ava II in the IVS2 of the β gene; and Bam H I 3’ to the β gene allowed to determine the restriction haplotypes that were numbered according to Orkin et al. [7].

Results

The proband was a 29-year-old high level athlete with a normal clinical presentation and normal haematological indices (table 1). In cation exchange HPLC, an abnormally high level of HbA1c (48%) has been noted with low HbA and normal HbA2 levels (figure 1). Since such a high level of HbA1c is incompatible with physiological possibilities, we suspected the presence of a hemoglobin variant coeluted with HbA1c. In order to identify this variant, we performed the molecular study of β-globin gene by DGGE and DNA sequencing.

The electrophoretic pattern obtained by DGGE clearly indicated the presence of a mutation on the third segment covering the third exon of the β-globin gene. Nucleotide sequencing of the amplified 367pb fragment of the β-globin gene, performed with forward primer revealed the presence of GGT→GAT substitution at codon 136 in exon 3 in heterozygous sate (figure 2), thus confirming that the hemoglobin variant coeluted with HbA1c was the Hb Hope [β136(H14) Gly→Asp (GGT→GAT)]. The same hemoglobin variant and mutation were also identified in the

### Table 1. Haematological data of the athlete with Hb Hope and her mother.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>RBC 10¹²/L</th>
<th>Hb g/dL</th>
<th>Hte %</th>
<th>MCV fl</th>
<th>MCH pg</th>
<th>MCHC g/dL</th>
<th>HbA %</th>
<th>HbA2 %</th>
<th>HbHope %</th>
<th>HbF %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proband</td>
<td>4.290</td>
<td>12.3</td>
<td>37</td>
<td>86.2</td>
<td>28.6</td>
<td>33.2</td>
<td>45.3</td>
<td>2.6</td>
<td>48.0</td>
<td>0.1</td>
</tr>
<tr>
<td>Mother</td>
<td>4.17</td>
<td>12.2</td>
<td>36.8</td>
<td>88.2</td>
<td>29.2</td>
<td>33.1</td>
<td>45.5</td>
<td>2.4</td>
<td>47.4</td>
<td>0.2</td>
</tr>
</tbody>
</table>
proband’s mother in heterozygous state. The hematological indices and Hb fractions of the mother are summarized in table 1.

Haplotype analysis showed the $\beta^{\text{Hope}}$ gene to be haplotype I (+—++) according to Orkin et al. [7].

Discussion

The present study reports the fortuitous description of Hemoglobin Hope (Hb Hope), $\alpha_2, \beta_2$ [136(H14) Gly$\rightarrow$Asp] at heterozygous state in a high-level Tunisian athlete. Other hemoglobinopathies such as thalassemia, HbS, HbC, HbD, HbG and HbOArab are more frequently encountered in Tunisia [8].

The Hb Hope is a $\beta$-globin variant resulting from a point mutation $\text{G}GT\rightarrow\text{GAT}$ at codon 136 of the $\beta$-gene. This abnormal Hb presented slightly faster migration than Hb A in an alcali pH buffer and has been described to be misjudged as increased glycohemoglobin [9] as noted in our study. Moreover, in other studies Hb Hope was reported to migrate like HbF on agar gel, near HbF on HPLC and with acetylated HbF on isoelectric focusing (IEF) [2].

The Hb Hope mutation has been shown to decrease the intrinsic oxygen affinity of the whole Hb to one-third as compared with that of HbA. This lower affinity for oxygen is beneficial to tissue oxygen delivery thus explaining the innocuous phenotype observed with this variant [2]. A reduced stability of Hb Hope has also been noted where its detection becomes difficult and compromised if samples are not analyzed in two or three days after blood collection.

Explanation of these altered functions were attempted on the basis of the altered tertiary structure of $\beta^{\text{Hope}}$ subunits [10, 11].

The Hb Hope was first identified by Minnich et al. in 1965 in a black African-American family in heterozygous state [12]. Later, the mutant was reported either alone or in association with Hb S[$\beta_6$ (A6) Glu$\rightarrow$Val (GAG$\rightarrow$GTG)] [2, 13, 14], Hb E [$\beta_2$ (B8) Glu$\rightarrow$Lys (GAT$\rightarrow$AAG)] [15], $\alpha$-thalassemia [16, 17] or $\beta$-thalassemia [18, 19] in several African-American, Japanese, Thais, Laotians, Mauritanian, Cubans and in Spanish families [18].

Recently, homozygous Hb Hope with Hb H disease was described in a Thai family [20]. All these descriptions raise the question of the origin of this variant that still remains unanswered. In this context, the role of polymorphisms and genetic markers co-inherited with hemoglobin variants – especially Hb S and HbC – and $\beta$-thal mutations is well known to be helpful for both molecular diagnosis and anthropological studies [7]; for this purpose, we took interest in the RFLP (restriction fragment length polymorphism) haplotypes associated with Hb Hope. Our results show that Hb Hope in this Tunisian family is found to be associated with the Mediterranean haplotype I (+—++). The same Hb Hope haplotype was reported in Thai [17, 21].

This favors that Tunisian and Thai Hb Hope would share a common Mediterranean origin, thus suggesting the possibility of a Mediterranean gene flow. However, in the absence of haplotype study in other Hb Hope reports, the question of a single or multiple origins of this variant remains unanswered.

Heterozygous Hb Hope (or compound Hb Hope-\(\alpha\)-thalassemia) is usually associated with an asymptomatic clinical state and a mild anaemia. This anaemia is most likely due to the combination of the unstable Hb Hope and the reduction in erythropoietin-mediated stimulus caused by the increased $O_2$ delivery to the tissues [2]. Accordingly, the HL athlete in this study showed an innocuous phenotype. She was unaware of her Hb status and her medical history was unremarkable and haematological data were in the normal range. The increase in $O_2$ delivery to the tissues has already been proposed as a possible selection pressure for Hb Hope due to the improvement of the pathological effects of certain hemoglobinopathies [2]. This increase in $O_2$ delivery may also result in another selection pressure for this variant, namely the improvement of physical aptitude,
work capacity and exercise tolerance, as demonstrated by the high level performances in tracks and field events in this athlete with Hb Hope.

Conclusion
This fortuitous description of Hb Hope in a Tunisian family showed a Mediterranean haplotype I, similar to that reported in Hb Hope of Thai families. However, the question of a single or multiple origins remains unanswered. On the other hand, the observation of Hb Hope in a high level athlete suggests a selection pressure of this Hb variant due to higher physical aptitude related to enhanced oxygen delivery to the tissues. This hypothesis will be verified using ergometry tests.

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Conflicts of interest: none.

References