

CASE REPORT

HBB mutations and HbA2 level: Escaping the carrier screening programs

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Abstract

HbA2 level alone for beta thalassemia trait may not be accurate and reliable even without iron deficiency so molecular genetic testing is important and should be considered for some individuals.

KEYWORDS

HbA2 level, *HBB* mutation, phenotypic variability, Thalassemia

1 | INTRODUCTION

Thalassemia's carrier screening programs are based on hematological parameters. However, some drawbacks mean not all mutations are detected. A clinical and genetic study of a *HBB* carrier is presented to show such a drawback. A family was referred to our center for genetic counseling. The pedigree was documented and the couple's hematological indices and HbA2 level were measured. *HBB* and *HBD* genes were sequenced. In silico analyses was then performed. Microcytosis, hypochromia, mild anemia, and minor thalassemia were noted, and the woman's HbA2 level was 3.3%. All individuals were carriers of an *HBB* mutation. A codon 82/83 mutation was found in the father, and the mother of the family was a carrier of an IVSII-1 (c.315 + 1G>A) mutation with MCH = 21.2pg and MCV = 96.6fL. The child was also carrier of IVS-1 with typical hematological indices. Given that HbA2 levels may vary, and some individuals may not

be detected in screening programs; we considered the role of modifier variants. That is the HbA2 marker may be insufficient for detection of beta thalassemia trait even without iron deficiency, and thus, molecular genetic testing is important.

Carrier and couple screening programs are the most successful way to diminish the incidence of thalassemia. Beta thalassemia is the most common cause of anemia worldwide.^{1,2} The prevention of thalassemia has been investigated for many years in Iran. Between 1990 and 1995, a control and prevention of thalassemia pilot program was undertaken. One of the major aims was to reduce the birth of new cases of the major thalassemia. During this program, the number of affected births decreased from 1,300 to 300 people per year, that is, the disease was prevented in approximately 80% of cases.³ Briefly, to detect carriers MCV, MCH, and HbA2 levels are screened in couples. If both members of the couple are detected as carriers, a molecular genetic diagnosis is recommended. Some people may have mild inherited anemia

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but HbA2 in normal range; sometimes these people are not identified in the screening process. In some cases, these individuals may have a complete deletion of the beta-globin gene cluster and if their husbands/wives have thalassemia trait, each pregnancy has a 25% chance of producing an affected child.

Different *HBB* mutations are found in different geographical areas. The highest incidence of the disease has been reported in Asia, particularly in residents of the coastal areas of South and Southeast Asian countries and regions around the Mediterranean.^{1,4} More than 900 *HBB* mutations have been characterized in different populations worldwide (<http://www.hgmd.cf.ac.uk/>). Several specific mutations (about 5-10 types) predominate and have a unique pattern of distribution for each population; for example, the six common mutations: c.315 + 1G>A, c.477 + 5G>A, c.92 + 6T>C, c.112delT, frame shift c.27_28insG, and c.251delG are responsible for 90% of all mutant alleles in the Mediterranean region.⁵ Numerical studies document genotype-phenotype correlations of patients with thalassemia.⁵⁻⁷

As an accurate and reliable marker for beta thalassemia trait, HbA2 is usually measured; it is normally between 2.2% and 3.5%. HbA2, a tetramer of alpha- and delta-globin chains, has been used as a diagnostic indicator of the presence of beta thalassemia trait in recent years. A relative surplus of alpha-globin chains in beta thalassemia trait individuals increases the formation alpha-delta dimer. In beta thalassemia trait, it is usually increased to more than 3.5%. HbA2 levels in β^0 mutation carriers reportedly are higher than in carriers of β^+ mutations. Hematological parameters in carriers of the c.315 + 1G>A mutation are as follow: HbA2 = 4.75% - 5.55%, MCH = 18.7 - 20.5 pg, and MCV 62.1 - 77.9 fL (<http://globin.bx.psu.edu/hbvar/menu.html>)⁸. But sometimes, HbA2 levels are not elevated. The most common cause of low

levels of HbA2 in beta thalassemia trait patients is concomitant of alpha thalassemia. In addition, low levels of HbA2 (usually between 35% and 40%) are observed in delta thalassemia, delta/beta thalassemia, and some delta-globin variants. Here, we report a Kurdish family with *HBB* mutation showing low level of HbA2 that passed the screening program and investigate the molecular basis of their condition.

2 | MATERIALS AND METHODS

2.1 | Clinical history and presentation

A couple (27-year-old woman and a 28-year-old man) with Kurd ethnicity was referred for counseling. The woman's hematological indices showed microcytosis, hypochromia, and mild anemia (Table 1) but the level of HbA2 was within the normal range (HbA2 = 3.3%). Based on the national program of thalassemia control guidelines,³ iron deficiency was diagnosed in the woman and iron therapy started. After 3 months of treatment with folic acid and ferrous sulfate, no change was observed in hematological indices.

2.2 | Genetic testing

A signed informed consent form was obtained; and five mL of peripheral blood was taken from the couple. DNA was extracted from using the conventional salting out method. The concentration of DNA was quantified using a spectrophotometer (NanoDrop ND2000c; Thermo Scientific). The coding and noncoding regions of the *HBB* and *HBD* genes were amplified and sequenced using forward and reverse primers [available upon request]. The polymerase chain

| Factor | Unit | Father | Mother | Son | Normal range |
|--------|-------------------|--------|---------|------|--------------------|
| RBC | millions/ μ L | 6.5 | 5.0 | 5.0 | 4.7-6.1 |
| Hb | g/dL | 13.1 | 10.7 | 11.3 | F:12-16 M:14-18 |
| MCV | fL | 64.0 | 69.6 | 64 | 80-96 |
| MCH | pg | 20.2 | 21.2 | 20 | 27-31 |
| MCHC | gm% | 31.6 | 30.5 | 31 | 33-37 |
| HbA | % | 92.5 | 90.1 | 91.5 | >97 |
| HbA2 | % | 5.8 | 3.3 | 5.8 | 1.5-3.5 |
| HbF | % | 1.7 | 6.6 | 2.7 | 0.0-0.9 |
| HCT | % | 41.5 | 35.1 | 36.7 | F:36-45 M:41-51 |
| SLI | % | 1292.8 | 1475.52 | 1280 | < 1530 |

TABLE 1 Hematological factors in this family

Abbreviations: Hb, Hemoglobin; HbA, Hemoglobin A; HbA2, Hemoglobin A2; HbF, Fetal Hemoglobin; HCT, Hematocrit; MCH, Mean Cell Hemoglobin; MCHC, Mean Corpuscular Hemoglobin Concentration; MCV, Mean Corpuscular Volume; RBC, Red Blood Cell; SLI, Shine and Lal index.

reaction (PCR) was carried out using a conventional protocol published elsewhere.⁸ Briefly, each reaction contains 50 ng of genomic DNA, 1 unit of Taq-DNA polymerase, 0.2 mM of dNTP, 0.2 mM of each primer, 1.5 mM of MgCl₂, and 1X PCR buffer. Thirty PCR cycles were as follows 95°C for 45 seconds, 67°C for 35 seconds, 72°C for 100 seconds, and 72°C for 10 minutes. The PCR products were directly sequenced by a BigDye termination method and use of a sequencing analyzer ABI 3500 (PE Applied Biosystems).

3 | RESULTS

Hematological indices in the woman were HbA₂ = 3.3 and HbF = 6.6, and in the man were HbA₂ = 5.8 and HbF = 1.7 (Table 1, Figure 1). Only in HBB gene, c.315 + 1G>A and c.251delG mutations were detected in the woman and the man, respectively. After genetic counseling and receiving required information about the risks of having children, prenatal diagnosis and; possible future difficulties, they decided to progress to marriage. During the pregnancy, the fetus was checked for *HBB* mutation. Fortunately, the fetus was not affected; it was diagnosed as a carrier of IVSII-1 (G > A). Their child was born. Hematological indices of the son are indicated in Table 1. The level of Hb A₂ was 5.8%. These two mutations were examined using HbVar Database in different populations (Table 2).

4 | DISCUSSION

Here is the family were not detected in the standard screening system. They presented having HBB mutations but normal HbA₂ levels. IVS II-1(G > A), c.315 + 1G>A, is a β⁰ type of mutation. In a few HBB mutation carriers with normal delta-globin genes, HbA₂ shows a normal level.^{9,10} In iron-deficient carriers of HBB mutation, a lower than expected HbA₂ has been reported^{11,12}; in a study conducted 444 people with HBB carrier, the mean HbA₂ was 5.3% and 5.6% in iron-deficient and non-iron-deficient individuals.¹³ Nevertheless, other investigations have explained no significant impact

of iron deficiency on the HbA₂ level in beta thalassemia carriers.^{14,15}

In the studied couple, hematological findings suggested that the man was a carrier but the woman was affected by iron deficiency-mediated anemia (Table 1). The SLI (Shine and Lal index) was less than 1530 for both individuals. Due to lack of response to treatment after two months in the woman, the HBB gene was sequenced and only a mutation c.315 + 1G>A was detected. Changes of hematological factors for carriers of the mutation are known (Table 3). In a study conducted in the Ilam population, the mean average of HbA₂ was equal to 5.4% in carriers of this mutation.¹⁶ But HbA₂ was only 3.3% in this woman, that is, it is borderline and in the normal range. This mutation leads to inactivation of donor splice site and disruption in normal splicing and processing of mRNA. The mutation was found for the first time in the Arab populations. c.315 + 1G>A is the most common mutation in Iran, Kuwait, and Yemen with a frequency of 26.47%, 29%, and 26.67%, respectively (<http://globin.bx.psu.edu/hbvar/menu.html>).¹⁷ The mutation shows a high frequency in most parts of Iran especially in central, western, and northern regions. Some mutations have an ethnic specific frequency; for example, a large deletion mutation in GJB6 is absent in Iranian populations¹⁸ or different mutations of CYP21A2 have a different distributions in our population.^{19,20}

The high HbF value (6.6%) of the woman may be due to a marked anemia. Furthermore, the gamma chains tend to compete with delta chains; therefore, there is a reduction in the real HbA₂ values that the beta zero thalassemia carrier would be expected to have. Phenotypic variability seen in this family may be due to modifier factors. Modifier genes have been reported in several genetic diseases.^{21,22} As known, the HBB has a central role in oxygen transport from the lung to the various peripheral tissues. In thalassemia, a number of genes have been reported as modifiers.²³⁻²⁵

The c.251delG (deletion of G at codons 82/83, AAGGGC) was reported for the first time in two Azerbaijanian patients.²⁶ But it is most prevalent in Czechoslovakian patients according to the HBVar database (7.53%). This variant is categorized as a β⁰ mutation.

A functional CACCC box at *HBD* promoter acts as the principal cause of the high activity of the *HBD* promoter, that is, absence of this box provides the binding site for the Krüppel-like factor 1 (*KLF1*) which acts as a transcription factor.^{27,28} It is predicted to modify pathological states of alpha-hemoglobin excess in diseases such as beta thalassemia.²⁹ In addition, other proteins such as cytoglobin may have a role during conditions of oxidative stress and in intracellular oxygen storage or transfer. The normal level of HbA₂ in the woman is probably due to a modifying variant in these genes. Further studies are recommended to find the variants of modifying genes; for example, the regulatory sequences of HBD and quantitative analyses of this gene could be done.

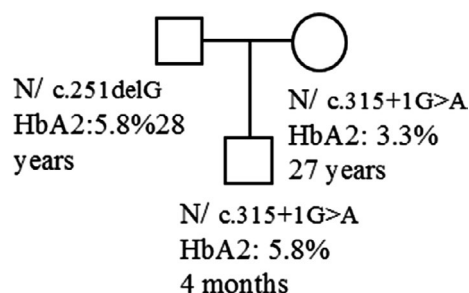


FIGURE 1 HBB mutations in the studied family. N: normal allele

TABLE 2 Distribution of these mutations in Iran and neighboring countries

| mutation | Iran (%) | Azerbaijan (%) | Turkey (%) | Kuwait (%) | Saudi Arabia (%) | Israel (%) | UAE (%) | Bahrain (%) | Oman (%) | Pakistan (%) |
|------------------|----------|----------------|------------|------------|------------------|------------|---------|-------------|----------|--------------|
| IVS II-1 (G > A) | 26.47 | 17 | 7.41 | 29 | 14.81 | 7 | 3 | 6.1 | 3.5 | 0.78 |
| Codon 82/83 (-G) | | 1.5 | 2.19 | | | | 1 | | | |
| Ref | 2 | 26 | 13 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |

TABLE 3 Hematological factors changes in carriers of IVSII-1

| Hematological factors | Laboratory findings |
|-----------------------|---------------------|
| Hb | 9.15 - 11.95 g/dL |
| HbA2 | 4.75% - 5.55% |
| Hb F | 1.75% - 4.45% |
| MCH | 18.7 - 20.5 pg |
| MCV | 62.1 - 77.9 fL |

So identifying the modifier genes is required for prediction of phenotype and may be helpful to interpret the unexpected results. Hence, molecular genetic testing should be performed to accurately diagnose beta thalassemia carriers.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

AS: did experimental procedures and wrote the draft of manuscript; NM: supervised, analyzed, and wrote the final draft. All authors: approved the final draft for submission.

RESEARCH INVOLVING HUMAN PARTICIPANTS AND/OR ANIMALS

All procedures were in accordance with the ethical standards of the Growth and Development Research Center ethical committee (IR.TUMS.CHMC.REC.1399.114) and with the 1964 Helsinki declaration standards.

INFORMED CONSENT

Informed consent was obtained from all individual participants included in the study.

DATA AVAILABILITY STATEMENT

There are no other data available.

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