CASE REPORT

First Report of Association Between Rare α -Thalassemia Mutation (*HBA1*: c.298A>T) and Hb Fontainebleau (*HBA2*: c.64G>C)

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Abstract α -Thalassemia is one of the most common monogenic diseases worldwide. The combination of alpha-chain variants with thalassemia mutations may lead to clinical and hematological characteristics, which is of importance for genetic counseling. The present study describes for the first time a rare $\alpha 1$ -globin nonsense mutation, codon 99 (*HBA1*: c.298A>T) associated with a $\alpha 2$ -chain variant Hb Fontainebleau (*HBA2*: c.64G>C) in a family from northern Iran. The case is a 23-year-old man with hypochromic microcytic anemia that requested for prenatal diagnosis. The combination of $\alpha 1$ -globin mutation and Hb Fontainebleau can cause clinical and hematologic features of thalassemia. This combination also highlights the important heterogeneity of alpha thalassemia in this part of the world.

Keywords α -Thalassemia (α -thal) · α -Globin gene · Mutation · Hb Fontainebleau

Elham Ghadami and Ahmad Tamaddoni have contributed equally to this work.

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Introduction

Alpha-thalassaemia is an autosomal recessive inherited disorder. It has clinical phenotype ranges from asymptomatic to a lethal hemolytic anemia that is caused by deletional and non-deletional mutations [5]. The non-deletional mutations are found less frequently than the deletional types. Based on gene frequency, $\alpha^{-3.7}$ and α^{-5NT} were the most common deletional and nondeletional mutations, respectively reported in Iran [9]. The structural hemoglobin variants are mainly due to point mutations in the α - or β -globin genes that cause single amino acid substitutions [6]. Hb Fontainebleau is a rare $\alpha 2$ chain variant caused by an alanine to proline substitution at codon 21 [4, 8], which has been previously reported in almost 24 cases around the world, four cases from Italy, two individuals from the UK, two from India and Iran, one from Cyprus, one from Iraq, and 12 cases from United Arab Emirates [3, 4, 7]. The α -globin mutations can affect mRNA processing, mRNA translation and globin chain stability [2]. Because the Iranian population is a mixture of different ethnic groups, frequency and distribution of α -globin mutations are different in various regions of this country. Thus, it is important to determine α -thal mutations and their associated phenotype in each ethnic group in order to facilitate genetic counseling, and hemoglobinopathies prevention. In this paper we report the first case of Hb Fontainebleau in association with a nonsense mutation (*HBA1*:c.298A>T).

Case Report

The patient, a 23-year-old male patient originating from Mazandaran Province in Northern Iran, who was diagnosed with hypochromic microcytic anemia was referred to the Amirkola Genetic Center, Babol, Iran, for genetic

Table 1 Hematological investigations

Parameter	Mother (Age 40)	Father (Age 45)	Proband (Age 23)	Reference range
RBC ($\times 10^{12}/L$)	4.65	5.09	6.14	4.10–5.10
Hb (g/dl)	11.9	14.3	13.6	12.3–15.3
MCV (fl)	78.1	84.1	67.3	87.6 ± 5.5
MCH (pg)	25.6	28.1	22.1	30.2 ± 2.1
MCHC (g/dl)	32.8	33.4	32.9	33–36
Hb A2 (%)	n.d.	n.d.	2.8	2–3%

RBC red blood cell, Hb hemoglobin, MCH mean cell hemoglobin, MCV mean cell volume, MCHC mean corpuscular hemoglobin concentration, n.d. not determined

counseling and mutation analysis for possible thalassemia prevention and prenatal diagnosis (PND) as his wife also presented with Hb D. The patients' parents were with normal (father) or mild anemia (mother) according to hematological indices, and no abnormal phenotype was observed. The hematological parameters of the studied family members are summarized in Table 1.

For identifying α -thal genotype, we investigated common deletional mutations, including $-\alpha^{3.7}$ (rightward), $-\alpha^{4.2}$ (leftward), $-(\alpha)^{20.5}$ and $(-)^{MED}$ by Gap-PCR. Then, we assessed 4 common point mutations in alpha globin gene [IVSI ($-5nt$) [GAGGTGAGG>GAGG—; HBA2:c.95p2_95p6delTGAGG], Hb Constant Spring [(Hb CS) (TAA > CAA) (HBA2:c.427 T>C)], Poly A2 (AATAAA > AATGAA; HBA2:c.*92A>G), and Poly A1 (AATAAA > AATAAG; HBA2:c.*94A>G)] by reverse dot blot and restriction site analysis. None of these mutations were observed in this case. Direct sequencing of the coding regions of $\alpha 1$ - and $\alpha 2$ -globin genes, *HBA1* and

HBA2 was performed in the next step. We found heterozygosity for a GCT > CCT at codon 21 of the $\alpha 2$ gene leading to substitution of Alanine → Proline, Hb Fontainebleau (Fig. 1).

Also, sequence analysis of $\alpha 1$ gene revealed a nonsense mutation (AAG > TAG, Lys → Stop) at codon 99 (*HBA1*: c.298A>T) (Fig. 2).

Discussion

Alpha thalassemia is typically caused by deletions of *HBA1* and *HBA2* genes. Non-deletional mutations account for a small proportion of cases of α -thalassemia [2]. Approximately 220 α chain variants that showed variable phenotypes with regard to the position of the mutation have been detected around the world [8]. The nonsense mutation on the $\alpha 1$ gene which causes the Lys → Term codon change that leads to production of a truncated protein was found in eight cases in North and South West of Iran. This mutation causes mild microcytic hypochromic anemia with normal Hb A2 levels [1]. Hb Fontainebleau is a rare alpha chain variant in the Iranian population. Two cases of this Hb variant had been reported in Khuzestan Province, Iran [3]. Hb Fontainebleau is associated with microcytosis without any other hematological defect [8]. The hematological index of the mentioned case was lower than that expected for α globin point mutation or Hb variant. Furthermore, his parents presented no symptoms of anemia (Table 1). Since family studies could not be carried out, we do not know the parental genotypes. However, regarding the hematological indices of the proband and his parents, we expect the two

Fig. 1 Sequencing results for $\alpha 2$ globin gene. **a** Heterozygous for Hb Fontainebleau [CD 99, Ala → Pro]; **b** normal sequence for $\alpha 2$ gene. [Codon-amino acid abbreviations: A (Alanine), H (Histidine), P (Proline), G (Glycine), E (Glutamic acid)]

Fig. 2 Sequencing results for $\alpha 1$ gene sequencing. **a** Heterozygous nonsense mutation [*HBA1*: c.298A>T]; **b** normal sequence for $\alpha 1$ gene. [Codon-amino acid abbreviations: V (Valine), N (Asparagine), F (Phenylalanine)]

mutations being in trans with a $\alpha^{\text{Fontainebleau}}/\alpha^{\text{Codon99}}$ genotype for the proband.

To the best of our knowledge, this is the first report of association between Hb Fontainebleau with a nonsense α -globin mutation. Our results confirm that the combination of thalassemia mutation with a Hb variant can cause clinical and hematologic features of thalassemia, which is important for genetic counseling and screening program, especially in regions of the world with high prevalence of thalassemia, and heterogeneous population. In fact, although in the present case each parent showed mild microcytosis with borderline hematological indices, but co-inheritance of an alpha globin chain variant with an alpha thalassemia mutation in the proband aggravated the severity of microcytosis leading to a typical hypochromic microcytic anemia.

In conclusion, identification of rare mutations is important for genetic counseling, and it can also help identifying carriers of such mutations in areas with a high prevalence of hemoglobinopathies through screening programs for carrier detection, and thalassemia prevention. Moreover, contrary to the populations with a hot spot mutation in which investigating 4–5 mutations allows to elucidate the molecular basis of anemia in most cases during screening programs, in heterogenous populations screening for prevalent mutations may not be sufficient. Rather, gene sequencing and phenotype-genotype correlation may be more successful, as some subjects may carry rare mutations or a combination of mild mutations.

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Compliance with Ethical Standards

Conflict of interest All the authors declares that they have no conflict of interest.

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