Identification of rare hemoglobin variant (Hb Fairfax) causing dominant β-thalassemia phenotype in an Iranian family.

Mohammad Taghi Akbari, Mohammad Hamid, Mina Izadyar

Ann Hematol
DOI 10.1007/s00277-010-1003-4

LETTER TO THE EDITOR

Identification of rare hemoglobin variant (Hb Fairfax) causing dominant β-thalassemia phenotype in an Iranian family

Mohammad Taghi Akbari · Mohammad Hamid · Mina Izadyar

Received: 25 April 2010 / Accepted: 17 May 2010

Dear Editor,

More than 900 hemoglobin (Hb) variants have been reported, and most variants are caused by mutations in the α- or β-globin gene clusters [1]. Clinically, most of hemoglobin variants are asymptomatic, but some variants are unstable, with altered oxygen affinity, or have a thalassemic phenotype (Hb Var database—http://globin.cse.psu.edu/globin/hbvar).

Hemoglobin Fairfax is a rare Hb variant, which has been reported only in an African-American child. This hemoglobin results from 15-base-pair tandem duplication of GAGCTGCACTGTGAC sequences inserted between codons 94 and 95, coding an additional Glu-Leu-His-Cys-Asp amino acids [2, 3].

M. T. Akbari
Department of Medical Genetics, Faculty of Medical Sciences,
Tarbiat Modares University,
Tehran 14115-111, Iran

M. T. Akbari
Tehran Medical Genetics Laboratory,
No. 297, Taleghane Street,
Tehran 1598619617, Iran

M. Hamid (✉)
Molecular Medicine Division, Biotechnology Research Center,
Pasteur Institute of Iran,
Tehran 13164, Iran
e-mail: hamid143@yahoo.com

M. Izadyar
Department of Pediatric Hematology,
Tehran University of Medical Sciences,
Markaze Tebbi Koodakan (Children Medical Center),
Tehran, Iran

We report on the hematological and molecular features of hemoglobin Fairfax for the first time in two members (mother and her daughter) of an Iranian family. This family was referred for prenatal diagnosis for β-thalassemia mutations.

Red blood cell indices and Hb analysis were carried out according to standard methods. Tests for unstable Hb using isopropanol precipitation showed positive results in both patients. After obtaining a written informed consent, molecular studies were conducted on genomic DNA isolated from peripheral blood cells by a salting out procedure [4]. For identifying α-thalassemia genotype, common deletional mutations were studied as previously described [5] with no mutation in α-thalassemia genotype. Triplication of α-globin genes was also excluded. Moreover, the entire β-globin gene was amplified and the DNA sequenced with the use of a primer set encompassing exons 1 and 2 (for fragment A: Beta1F 5′-GGGCCAAGAGATA TATCTTAG-3′, Beta1R 5' AATGACATGAACTTA ACCATAG-3′) and another encompassing exon 3 (fragment B: Beta2F 5′-GCAC CATTCTAAAGAATAACAG-3′, Beta2R 5′-GTTTGAAC TAGCTCTTCATTTC-3′). The sequencing reactions were performed by the chain termination method on ABI 3730 XL sequencer (Primm, Milan, Italy), as described elsewhere [6, 7]. The nucleotide numbering is based on GenBank accession number U01317.

The hematological and molecular features of hemoglobin Fairfax in the index patient and her mother aged 5 and 29 years old, respectively, are summarized in Table 1.

The index case with severe hemolytic anemia, marked splenomegaly, and blood transfusion dependency (every 20 days) receives iron chelating therapy (Desferal) four times a week. She had no other thalassemic features.

Published online: 03 June 2010

Table 1 Hematological and molecular data of the mother and offspring with the Fairfax variant

Hematological index	Mother, post-transfusion	Offspring, pre-transfusion	Offspring, post-transfusion
Hb (g/dl)	8.9	6.3	9.0
RBC (10^{12}/l)	2.96	2.25	3.28
HCT (%)	34.2	23.6	26.7
MCV(fL)	115.5	104.7	81.4
MCH (pg)	30.1	28	27.4
MCHC (%)	26	26.7	33.7
Hb A (%)	83.4	?	95.4
Hb F (%)	13.1	?	1.8
Hb A2 (%)	2.7	?	2.8
α-Genotype	αα/αα	αα/αα	αα/αα
β-Genotype	Insertion (GAGCTG CACTGTG AC)/normal	Insertion (GAGCTG CACTGTG AC)/normal	Insertion (GAGCTG CACTGTG AC)/normal

The mother underwent splenectomy during childhood at age 7, was dependent on blood transfusion (every 2–3 months), receiving Desferal four times a week, and had two miscarriages before giving birth to this child. It is interesting to note that in both missed pregnancies the hemoglobin level had dropped to 3–4 g/dl as a result of non-transfusion. It is highly probable that fetal deaths have occurred due to hypoxia. Therefore, the clinical feature of both patients are in favor of β-thalassemia major.

This is the first report from an Iranian family and the second one in the world to the best of our knowledge. This family was from Tonokabon, north of Iran with Persian ethnic origin.

In the abovementioned patients, a 15-base-pair tandem duplication of GAGCTGCACTGTGAC sequences was detected in exon II by direct sequencing in heterozygous status. The sequence data of this insertion is shown in Fig. 1. As previously reported, the duplication/insertion mutation in the heterozygous state has also been associated with β° thalassemia phenotype. As for the molecular mechanism of how this kind of mutations occur, the reported mutation is caused by a backward replication slippage due to the existence of a 4-bp repeat on both flanking regions of the inserted fragment [8]. Nevertheless, the Fairfax mutation must have been caused by a different mechanism because it lacks homologies in the flanking regions. This variant is due to five amino acids inserted between codons 94(FG1) and 95(FG2) of β chain. Because of duplication in this region, it is possible to make changes in the binding of F8 proximal histidine to heme, which might result in the rearrangement of the heme pocket, leading to an unstable variant.

To date, only one other duplication/insertion of 15 nucleotides, Leu-His-Cys-Asp-Lys inserted between codons 95(FG2) and 96(FG3) of beta chain (slightly different from our case), has been described. This unstable hemoglobin variant was found in a Japanese girl with very severe, chronic hemolytic anemia (Hb Var database—http://globin.cse.psu.edu/globin/hbvar) [9].

In conclusion, it is worth emphasizing that this Fairfax Hb variant could be identified by a molecular technique because it is unstable and not detectable by conventional hemoglobin electrophoresis.

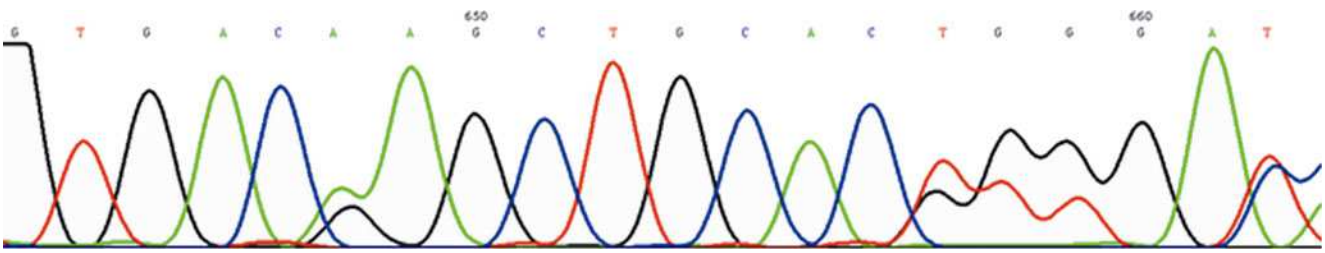

Fig. 1 β-Globin gene sequencing showing the15-base-pair tandem duplication (GAGCTGCACTGTGAC) inserted between codons 94 and 95

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