

Report of Haemoglobin J-Toronto and alpha thalassemia in a family from North of Iran

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Abstract

We report of an Iranian family with history of a rare haemoglobin variant, Haemoglobin J associated with alpha thalassemia, discovered while performing premarital thalassemia screening. In the present study we report the first case of haemoglobin J-Toronto [α 5 (A3) Ala>Asp] on α -globin gene, found in a 16-year-old female from Mazandaran Province, North of Iran. Further investigation characterized the same mutation for mother and brother of the proband, whilst mother was also a carrier of an alpha thalassemia gene mutation ($-\alpha^{3.7}$). Haemoglobin J-Toronto was previously just reported from Canada and has not been found in any part of Iran.

Keywords: Haemoglobin J variant, α -globin gene, Rare mutation, Northern Iran.

Introduction

There are hundreds of different variants of haemoglobin caused by structural alteration of alpha, beta or gamma globin chains varying from amino acid replacements, elongated chain, deletions, insertions, or both deletions and insertions. Abnormality of beta-chain or alpha-chain produces most of the clinically significant haemoglobinopathies. The status of zygosity also plays a very important role in the expression and deletion of the disorder. In heterozygous variants the other normal allelic gene produces normal chains which may compensate for the defective gene. In the homozygous state, both allelic genes

are affected which results in the production of a large amount of the variants.

Haemoglobin J (Hb-J) was first described by Thorup et al. in an African-American patient (1956)¹ and since then more than 50 variants of Hb-J such as Hb-J Capetown [α 92(FG4) Arg>Gln], Hb J-Buda [α 61(E10) Lys>Asn], Hb J-Chicago [β 76(E20) Ala>Asp], Hb J-Sardegna [α 50(CE8) His>Asp], and Hb J-Toronto [α 5(A3) Ala>Asp] are identified. In comparison with mature haemoglobin, these haemoglobins generally show faster movements than haemoglobin "A" on cellulose acetate electrophoresis (i.e. closer to the anode). Hb-J variants correspond with certain single or multiple base changes in haemoglobin alpha or beta chains; however there are some Hb-J subtypes with more than one amino-acid replacement in alpha chain (e.g. Hb J-Singapore).² One recently described Hb-J, found in a few members of an Italian family, named Hb J-Europa has a beta chain substitution [β 62(E6) Ala>Asp].

Some of Hb-J variants have abnormal properties and affect respective haematologic indices, whilst majority of them do not result in any abnormal clinical manifestation. For instance, Hb J-Cape Town, the most commonly seen Hb-J variant, in heterozygous case is associated with increased oxygen affinity and polycythemia. Those affected may also have a mild erythrocytosis and microcytosis. Other variants like Hb J-Sardegna and Hb J-Oxford will show a completely unremarkable clinical picture in the heterozygote patients. The Hb-J gene follows the pattern of autosomal codominant

inheritance. The existence of Hb-J does not affect the longevity or survival of the foetus.

Case Report

For premarital thalassemia screening, a 16-year-old Iranian female with Gilak ethnicity was admitted to Fajr laboratory center, Sari. All evaluated haematologic indices were in the normal range (Table); however the variant band was seen in the location of Hb-J (a fast migrating variant) on isoelectric focusing (IEF) and cellulose acetate electrophoresis. The variant constituted approximately 22.5% of total haemoglobin. Further haematological investigation was performed for the whole family. The father's blood picture and haemoglobin electrophoretic pattern were completely normal. The mother was 35 years old with an Iranian origin and born in Ghaem-Shahr in the North Coast of Iran. She had low MCV (76.5 fL) and MCH (25.1 pg) indices, but normal HbA2 levels. The 11-year-old brother of the case had a history of hypochromic microcytic anaemia while clinical examination was normal (Table). In

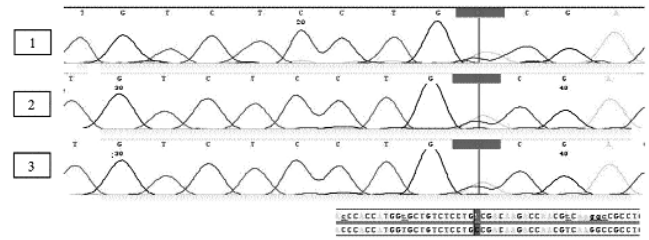


Figure: DNA sequence analysis of the alpha 1 gene. The coding exon DNA from the proband, the mother and the brother were amplified by PCR and sequenced. Segments of nucleotide sequences containing mutation for the brother (1), the proband (2) and the mother (3) are shown. The position of the single C to A missense mutation (c.17C>A; GCC>GAC at codon 5) is indicated by colored box.

mentioned alpha globin gene deletion, suggesting that the two mutations are localized in trans position on mother's two different haplotypes, and her possible genotype is $-\alpha^{3.7}/\alpha J$. Coexistence of two different types of haemoglobinopathies affected mother's haematological indices. This finding is similar to the results of the work of Old and colleagues, where combination of a certain type of Hb-J (Hb J-Tongariki) and

Table: Hematologic indices and haemoglobin fractions of the examined cases.

	Sex	Age (year)	RBC (x 10 ⁶ /μl)	Hb (g/dL)	Hct (%)	MCV (fL)	MCH (pg)	MCHC (%)	Hb-A (%)	Hb-A2 (%)	Hb-F (%)	Hb-J (%)
Father	M	41	5.89	14.8	43.6	74	25.1	33.9	96.9	2.6	0.5	0
Proband	F	16	5.12	13.3	40.1	78.3	25.9	33.1	75.1	2.2	0.2	22.5
Brother	M	11	5.17	12.3	38.1	73.7	23.7	32.2	72.3	2	0	25.7
Mother	F	35	5.63	14.1	43.1	76.5	25.1	32.7	70.4	1.8	0	27.8

haemoglobin electrophoresis of the two samples donated by mother and brother of the proband, Hb-J was detected. Hb-J identity was confirmed using automatic capillary zone electrophoresis (Minicap system; Sebia, France).

The family was referred to the Kariminejad-Najmabadi Pathology and Genetics Center, for further molecular analysis. DNA samples were obtained from all members of the family and polymerase chain reaction (PCR) was performed according to the protocol described by Baysal and Huisman³ for the detection of $-\alpha^{3.7}$, $-\alpha^{4.2}$, and $-\alpha^{MED}$ mutations as common alpha thalassemia gene mutations in Iran.⁵

Results of the test revealed that the mother is a carrier of alpha globin gene deletion ($-\alpha^{3.7}$). The presence of this deletion and following Hb A content reduction, resulted in the increase of Hb-J percentage above 25% of the total haemoglobin content. Same pattern was observed in the brother's case. It was concluded that he is also a carrier of an alpha chain gene deletion, yet different than the evaluated mutations.

While the two children inherited Hb-J abnormality from their mother, none of them were carriers of the

alpha thalassemia robustly affected the red cell indices.⁵

Samples were further analyzed by sequencing 1 and 2 chains. Using the ABI PRISM™ Big Dye Terminator Cycle Sequencing kit and the ABI PRISM™ 3100-Avant Genetic Analyzer (Applied Biosystems, USA) cycle sequencing was done. Sequencing of α -globin genes revealed single C to A missense mutation (GCC>GAC) at codon 5 in the α -globin gene in all three suspected cases (Figure). Molecular analysis of β -globin genes in suspected cases was performed according to our previously reported method in Iranian β -thalassemia patients.⁶ Sequencing of β -globin genes revealed no β thalassemia mutation in indexed case as well as the other samples.

Discussion

The first report of finding any Hb-J variant in Iran (Hb J-Iran; ?2?2 His >Asp) dates back to 1967.⁷ The same scientist has reported of Hb J-Kurosh [alpha 19 (AB) Ala>Asp] in 1976 from Iran.⁸ Regarding the mutations found in the neighboring countries and Asia, Hb J-Tashikuergan [alpha 19 (AB1) Ala>Glu] has been reported in populations

in the Silk Road region of China.⁹ Another Hb-J variant, Hb J-Ankara [beta 10 (A7) Ala>Asp], has been reported from Turkey in 1974.¹⁰

Province of Mazandaran in North of Iran is a strip of land located between Alborz Mountains and Caspian Sea. Thalassemia and different types of haemoglobinopathies are commonly reported in this area, yet this is the first report of existence of such Hb-J variant in combination with alpha thalassemia in Iran. As previous reports of such haemoglobinopathy were from occidental countries, it would be interesting to carry out a molecular study to find out whether there is a common origin for this mutation and any relation among these separate cases reported from around the world.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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