First Report on the co-inheritance of (β) IVS I-1 (G→T) Thalassemia with the (γ) CD85 [Phe→Ser (F1) (TTT→TCT)] HbA2 Etolia in Iran

Beta thalassemia minor phenotypes with normal HbA2 levels and decreased MCV and MCH values are relatively rare beta-thalassemia traits. Here, we describe a case with normal HbA2 and decreased MCV and MCH level. Amplification refractory mutation system-polymerase chain reaction (ARMS-PCR) revealed IVS I-1 (G→T) mutation in the beta-globin gene. Direct sequencing of the delta-globin gene revealed a previously reported Hb variant called Hb A2 Etolia (Gene Bank Accession No. DQ106871). This is the first case reporting HbA2 Etolia in association with the beta-IVS I-1 (G→T) mutation in Iran. Reduced HbA2 expression by a co-inherited HbA2 variant resulting in decreased HbA2, in Cis or Trans, may cause problems in carrier diagnostics and eventually in genetic counseling and prenatal diagnosis when insufficient molecular analyses are performed.

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Most of the common forms of β-thalassemia (thal) are associated with increased level of HbA2 in heterozygotes. However, HbA2 level is in normal range for the varieties such as δβ-thal, γδβ-thal, αβ-thal and some β-thal with mild beta globin gene mutations (i.e. Mild and Silent β-thal). The most common cause of normal HbA2 is the co-inheritance of both β- and δ-thal.[1] The mutations of the δ-globin gene, that decrease the quality or quantity of γ-globin chain synthesis solely, have no clinical effect. However, γ-thal defects may reduce the otherwise elevated levels of Hb A2 in β-thal traits thus compromising the diagnosis.[1-3] Here we report co-segregation of a Beta globin gene mutation with the Hb A2-Etolia mutation which was first described in a Greek family in association with HbA2-Pylos [δ 11(A8) Gly→Val] by Drakoulakou et al. in 1997.[3]

Genetic counseling for every couple, who have β-thal hematological data [i.e. low MCV (<80 fL) or MCH (<27pg) and high Hb A2 (>3.5%) levels], has become compulsory in Iran since 1997. Then depending on couple's decision, they will be referred for further investigation including carrier detection and prenatal diagnosis.[4] A 37-year-old man, presenting with hypochromic microcytic anemia, and his spouse were referred to our laboratory for carrier detection and prenatal diagnosis. Complete blood count (CBC), alkaline hemoglobin (Hb) electrophoresis and cation exchange high performance chromatography (HPLC) using the Hb Gold System (Drew Scientific, Cumbria, UK) in several repeated tests, revealed low MCV and MCH level for the proband and his wife (Table 1). The woman presented with a typical high Hb A2 β-thal carrier phenotype, but the husband repeatedly showed normal HbA2 levels. Hematological data of the family members are summarized in Table 1. The proband had a higher RBC level in comparison to his wife. RBC level is slightly higher in males, however, many factors such as smoking and hypo-hydration might have led to this increased RBC level.

Both family members were tested for β-thal mutations at the DNA level using the amplification refractory mutation system-polymerase chain reaction (ARMS-PCR) method.[5] ARMS-PCR identified IVS I-1 (GdT) and IVS I-6 (T→C) for the man and his wife, respectively.

Table 1. Hematological Data of the Family. 1-Total Iron Bin.Cap, 2-The data was not applicable.

	Proband	*Spouse*	*Proband's Father*	*Proband's Mather*
Found Mutation	δ CD85 (TTT→TCT) & IVS I-1 (G→T)	IVS I-6 (T→C)	δ CD85 (TTT→TCT)	IVS I-1 (G→T)
Sex-age	M-37	F-28	M-63	F-59
Ferritin ng/mL	118	49	---2	---
Iron ug/dL	63	56	---	---
TIBC1 ug/dL	265	251	---	---
Hb (g/dL)13.4	12.0	14.5	10.9	
RBC (10^{12}/L)	7.2	5.4	5.5	5.56
MCV (fL)61.0	69.0	83.9	63	
MCH (pg)19.0	22.0	26.2	19.6	
MCHC (g/dL)	31.0	32.0	32	31.2
Hb A (%)96.9	92.0	97.8	94.6	
Hb A2 (%)2.6	3.5	1.6	4.8	
Hb F (%)0.5	4.5	0.6	0.6	

The proband's Fe, Ferretin and TIBC levels were at normal range (Table 1). Also, our try to find any mutation in alpha globin genes of the proband had no results using direct sequencing and multiplex-PCR.[6] Since the most common cause of normal HbA2 is the co-inheritance of β- and δ-thal,[1] DNA sequencing of delta globin gene performed for proband and his parents to assess the cause of the normal Hb A2 level in the proband carrier of the β0 IVSI-1(G→T) mutation. Two specific primer sets were designed. The first set amplifies a 733 bp fragment on the δ-globin gene including exon I, exon II and their flanking regions on the delta globin gene. The sequences of these primers were: δ1F 5'-AGG GCA AGT TAA GGG AAT AGT G-3' and δ1R 5'-ATG ACA AAA ATG TGG GAG AAG AG-3'. The second primer set amplifies a 458bp fragment including exon III and its flanking regions on the δ-globin gene. The sequences of these primers were: δ2F 5'-TGG GTG TTG GCT CAG TTT CTC-3' and δ2R 5'-GCT TTT CTC TTT TCC CAT GTA CTC-3'. Sequence analysis revealed the δ CD85 (TTT→TCT, Phe>Ser) HbA2-Etolia mutation in a heterozygous state both in the proband (Figure 1) and his father. Since the HbA2-Etolia mutation and the β^0 IVSI-1(G→T) mutation were in the father and mother of the proband, respectively, the proband mutations were not segregated by a single chromosome to the proband. Thus the proband mutations are in Trans.

The Hematological and hemoglobin analysis data for HbA2-Etolia mutation presents normal indices, except for the reduced HbA2 level. The HbA2-Etolia mutation is localized at the helical positions F1 of the HbA2, thus it potentially causes molecular instability of the tetramer and leading to reduced HbA2 percentage.[3]

There are several earlier reports on the co-inheritance of different δ-globin gene mutations with β-globin gene mutations in other populations (3, 7-9). Our case is the first report on the co-inheritance of γ CD85 (TTT→TCT) with the β^0 IVS I-1 (G→T) mutation. This case and our recent report on the co-incidence of HbA2 Troodos with the IVS-II-1 (G→A) β0-thalassemia,[7] suggest that the frequencies of delta-chain variant might be high in Iranian populations.

Phenotypes associated with low MCV and MCH and normal Hb A2 levels could be induced by different geno-

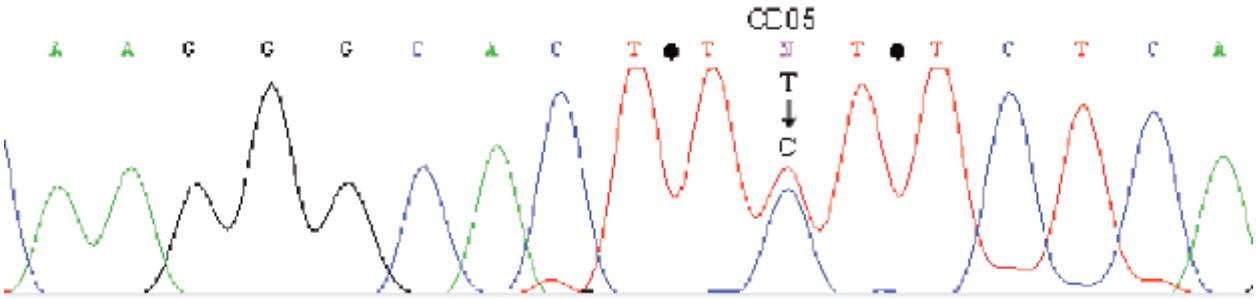

Figure 1. The direct sequence analysis result of the 733bp fragment of δ-globin gene. The T→C substitution at the first base of the codon 85 is indicated with N.

types (i.e., α-globin carrier status, δ/β gene deletion, etc.). Therefore, molecular characterization of the underlying cause is very important. Failure to do so may cause potential pitfalls in genetic counseling due to problems in molecular diagnosis. This is true in countries like Iran where thalassemia is widespread and heterogeneous from region to region. It is especially important when an extensive national program is underway to offer the choice of prevention to informed couples at-risk and thereby reduce the incidence of births affected with severe forms of hemoglobinopathies (S. Zeinali, personal communication).

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