Hb Knossos: HBB:c.82G>T Associated with HBB:c.315+1G>A Beta Zero Mutation Causes Thalassemia Intermedia

Hengameh Nasouhipur · Ali Banihashemi ·
Reza Youssefi Kamangar · Haleh Akhavan-Niaki

Received: 23 August 2013 / Accepted: 15 January 2014 / Published online: 31 January 2014
© Indian Society of Haematology & Transfusion Medicine 2014

Abstract β -thalassemia is the most common single gene disorder worldwide and in Iran. In the present study we report for the first time a rare variant of hemoglobin HBB:c.82G>T; Codon 27 GCC→TCC (Ala→Ser), Hb Knossos, using sequencing and reverse dot blot hybridization, in members of a family from North Iran. The family has a 16 years-old compound heterozygous thalassemia intermedia male child presenting this variant together with HBB:c.315+1G>A (IVSII-I) mutation. The father, heterozygous for Hb Knossos, showed borderline hematological indices. To our knowledge, this is the first report of Hb Knossos in trans with the β^0 IVSII-I allele leading to thalassemia intermedia. Our data also highlight the necessity of deep molecular characterization of subjects presenting normal HbA2 level associated with abnormal or borderline red cell indices.

Keywords Thalassemia intermedia ·
Hb Knossos · Iran

Introduction

β -thalassemia is a recessive monogenic disorder encountered worldwide with a higher prevalence among Mediterranean, Middle Eastern and Indian populations [1]. The disease is due to mutation in β globin locus for which more than 200 alleles have been reported [2]. Carriers present mild anemia while affected patients have severe anemia and require regular transfusion. Intermedia phenotype with occasional or no transfusion might also occur in patients who inherited mild β globin mutations or in the presence of modulators. Hemoglobin disorders are highly prevalent among Iranians specially those originating from northern and southern provinces [3–5]. Northern Iranian β -thalassemia traits are genetically highly heterogeneous and more than 25 β globin gene mutations were described in this population [3, 4].

In this report, we describe a rare hemoglobin variant caused by a mutation in β -globin gene, HBB:c.82G>T: Codon 27 GCC→TCC (Ala→Ser), Hb Knossos, which produces the classical phenotype of intermedia β -thalassemia in association with HBB:c.315+1G>A (IVSII-I) β^0 thalassemia trait.

Hb Knossos was first described in a Greek family in which thalassemia intermedia was diagnosed for three members who were compound heterozygous for a typical β -thalassaemia and this variant [6]. It was demonstrated that some β Knossos RNA transcripts utilize a cryptic splice sequence and are therefore abnormally processed [7] and the amount of the variant in carriers is approximately 30 % of the total hemoglobin [8].

In heterozygous state, the mutation results in mild β -thalassemia phenotype with a normal HbA2 level, whereas the homozygous state results in intermediate β -thalassemia [9, 10]. It's slightly decreased oxygen affinity may account for the observed phenotypes [11].

H. Nasouhipur · H. Akhavan-Niaki (✉)
Cellular and Molecular Biology Research Center, Babol
University of Medical Sciences, Babol, Iran
e-mail: halehakhavan@yahoo.com

H. Nasouhipur
e-mail: hnasouhipur@yahoo.com

A. Banihashemi · R. Youssefi Kamangar · H. Akhavan-Niaki
Genetic Laboratory of Amirkola Children Hospital, Babol
University of Medical Sciences, Babol, Iran
e-mail: sina2222@yahoo.com

R. Youssefi Kamangar
e-mail: R.Yosefi.Kamangar@gmail.com

Table 1 Hematological features of members of a family with Hb Knossos mutation

	Relation	Hb (g/dl)	MCV (fl)	MCH (pg)	Hb A2 %	HbF %
1	Father	13.4	80	26.7	1.8	0.9
2	Mother	10.9	69.2	21.5	4.2	1.6
3	Proband (boy)	8.1	64.5	21.5	1.3	31.6

Fig. 1 Hb Knossos point mutation in heterozygous form in the investigated subject

This rare variant was previously reported in North African (Egypt, Algeria, Tunisia, Libya), Mediterranean (Greece, Turkey) and Middle Eastern (Jordan) populations. The highest frequency for this mutation was observed among Jordans with 3.3 % prevalence while a frequency less than 1 % was reported for others [12].

To our knowledge this variant has not been previously reported among Iranians. Moreover the combination of this hemoglobin variant with HBB:c.315+1G>A (IVSII-I) β^0 mutation and its associated phenotype is described for the first time.

Case Report

The case is a 16 years old boy originating from north Iran, Mazandaran province, with severe microcytosis and hypochromia and elevated HbF who so far has not been transfused and is consequently considered as thalassemia intermedia. His father is 38 years old with no abnormal phenotype and borderline hematological indices. His mother is 35 years old with hematological indices typical of β -thalassemia trait (Table 1).

Molecular analysis of previously reported β globin point mutations in this province were screened by reverse dot blot method for all three members of the family and so far only HBB:c.315+1G>A (IVSII-1) was detected in the

patient and his mother. Finally, direct sequencing of amplified DNA of the β globin gene of the young boy revealed a second mutation at codon 27 of exon 1 with GCC→TCC (Fig. 1), causing an Ala→Ser substitution in β globin chain; HBB:c.82G>T. The presence of this point mutation which leads to the synthesis of Hb Knossos variant [$\alpha_2\beta_2$ 27(β_9) Ala→Ser] was also confirmed at the DNA level in the father by a home-made reverse dot blot assay.

Molecular analysis of alpha chain was also performed and none of the alpha-globin gene deletions or point mutations commonly reported in Iran [12] were detected in this patient.

Discussion

Despite the abundance of β globin alleles in the world, most populations present a limited spectrum of these mutations and in the majority of cases five to ten different mutations cover more than 90 % of mutant alleles. In Iran, due to the high heterogeneity of the population, more than 60 different β globin gene mutations were reported and there are still new cases reported in the recent years highlighting the genetic complexity of thalassemia in this part of the world.

Hb Knossos, β 27 (B9) Ala→Ser [HBB:c.82G>T] is a rare Hb variant in the world present in North Africa and Mediterranean region at a frequency lower than 1 % and with a relatively higher prevalence in Jordan (3.3 %) [13]. To our knowledge, this is the first time this mutation is reported in Iran. As Hb Knossos was also rarely reported in Turkish population [14–16], the western neighbor of Iran, we hypothesized that this mutation might have spread over the Middle Eastern and Mediterranean countries. Our data also confirm that compound heterozygosity for Hb Knossos and a β^0 mutation [IVSII-1 (G-A)] does not cause severe transfusion dependent thalassemia. This is again the first time such allelic combination is reported. Other combinations with either β^0 codon39 (C→T) [10], C8(–AA) [14], or β^+ [–30 T-A, –101 (C-T), IVSI-5 (G-A) or IVSI-110(G-A)] alleles [15] leading to thalassemia intermedia with different degrees of severity were reported in other parts of the world while a case of thalassemia major compound heterozygous patient harboring a β^+ mutation [IVSII-745(C-G)] was also reported [16]. Taken together these phenotypic variations suggest that other genetic components might be implicated in determining the severity of anemia in compound heterozygotes.

This report provides a further piece of evidence of the high heterogeneity of β globin mutations in Iran and the hematological features of the proband which reveal the hemoglobin Knossos as a silent mutation highlight the necessity of deep molecular characterization of subjects

presenting normal HbA2 level associated with abnormal or borderline red cell indices during premarital screening specially when the partner is a typical β thalassemia trait in order to reach a better genetic counseling and control of severe anemia child births.

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

References

1. Weatherall DJ (1991) The thalassemias. In: Williams JW (ed) Haematology, 6th edn. McGraw Hill, New York
2. Weatherall DJ, Clegg JB (2001) The thalassaemia syndromes. Blackwell Science, Oxford
3. Akhavan-Niaki H, Derakhshandeh-Peykar P, Banihashemi A et al (2011) A comprehensive molecular characterization of β thalassemia in a highly heterogeneous population. *Blood Cells Mol Dis* 47(1):29–32
4. Derakhshandeh-Peykar P, Akhavan-Niaki H, Tamaddoni A et al (2007) Distribution of β -thalassemia mutations in the northern provinces of Iran. *Hemoglobin* 31(3):351–356
5. Najmabadi H, Karimi-Nejad R, Sahebjam S et al (2001) The beta-thalassemia mutation spectrum in the Iranian population. *Hemoglobin* 25(3):285–296
6. Fessas PH, Loukopoulos D, Loutradi-Anagnostou A, Komis G (1982) “Silent” beta-thalassemia caused by a “silent” beta-chain mutant: The pathogenesis of a syndrome of thalassemia intermedia. *Br J Haematol* 51(4):577–583
7. Orkin SH, Antonarakis SE, Loukopoulos D (1984) Abnormal processing of beta Knossos RNA. *Blood* 64(1):311–313
8. Arous N, Galacteros F, Fessas P et al (1982) Structural study of hemoglobin Knossos, beta 27 (B9) Ala leads to Ser. A new abnormal hemoglobin present as a silent beta-thalassemia. *FEBS Lett* 147(2):247–250
9. Baklouti F, Dorléac E, Morlé L et al (1986) Homozygous hemoglobin Knossos (alpha 2 beta 227(B9) Ala-Ser): a new variety of beta (+)-thalassemia intermedia associated with delta (0)-thalassemia. *Blood* 67(4):957–961
10. Sahli CA, Bibi A, Ouali F et al (2012) δ 0-thalassemia in cis of β Knossos globin gene: first homozygous description in thalassemia intermedia Libyans and first combination with codon 39 (C→T) in thalassemia intermedia Tunisian patients. *Clin Chem Lab Med* 50(10):1743–1748
11. Fessas P, Loukopoulos D, Kokkinou S et al (1986) Hemoglobin Knossos: a clinical, laboratory, and epidemiological study. *Am J Hematol* 21(2):119–133
12. Akhavan-Niaki H, Youssefi Kamangari R, Banihashemi A et al (2012) Hematologic features of alpha thalassemia carriers. *Int J Mol Cell Med* 1(3):162–167
13. Zahed L (2001) The spectrum of beta-thalassemia mutations in the Arab populations. *J Biomed Biotechnol* 1(3):129–132
14. Kutlar A, Kutlar F, Aksoy M et al (1989) Beta-thalassemia intermedia in two Turkish families is caused by the interaction of Hb Knossos [beta 27(B9)Ala-Ser] and of Hb City of Hope [beta 69(E13)Gly-ser] with beta (0)-thalassemia. *Hemoglobin* 13(1):7–16
15. Altay C, Gürgey A (1990) Beta-thalassemia intermedia in Turkey. *Ann NY Acad Sci* 612:81–89
16. Keser I, Manguoglu E, Kayisli O et al (2007) Combination of Hb Knossos [Cod 27 (G-T)] and IVSII-745 (C-G) in a Turkish patient with beta-thalassemia major. *Genet Test* 11(3):228–230