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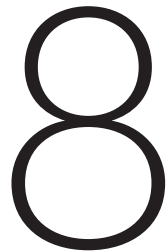
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CHAPTER

KNOWN AND NEW δ GENE MUTATIONS AND OTHER FACTORS INFLUENCING HBA2 MEASUREMENT IN THE OMANI POPULATION

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ABSTRACT

Although delta thalassemia is not categorised as a severe disease, it is essential to know the molecular spectrum of the delta gene mutations frequently occurring in specific areas in particular if these areas are characterized by a high rate of beta thalassemia such as Oman. This is because co-inherited delta globin gene defects can interfere with the basic diagnosis of β -thalassemia carrier when this is based upon the measurement of the HbA₂ only. For that, we have investigated 33 patients with low HbA₂ levels collected from different hospitals in Oman. Some cases had a second HbA₂ fraction, while others had only significantly lower HbA₂ levels. Among these patients, 20 did carry a δ -globin gene mutation, the rest were carrier of alpha thalassemia defects or could be iron depleted or both. In total, eight different known mutations and 2 novel delta determinants were found. The characterization of the δ -gene mutation spectrum will improve carrier diagnostics and genetic counseling in the Omani population screened for beta thalassemia.



INTRODUCTION

After the age of two, postnatal haemoglobin A (HbA) is the major haemoglobin component of the red cells. Besides HbA and in normal conditions, about (2.5-3.5%) of the haemoglobin content will consist of haemoglobin A₂ (Hb A₂) while traces of HbF (<1%) will present in adult life (1). Mutations that occur in the δ -globin gene (HBD, MIM# 142000) can affect the structure or the expression of the delta globin chain as it is the case for all other globin genes. Structural defects, if stable, will produce a second and usually visible Hb A₂ fraction (2). If unstable, the mutation will behave as a thalassaemic defect and be undetectable using basic methods such as high performance liquid chromatography (HPLC) or capillary electrophoresis (CE). Thus, DNA analysis will be required to differentiate between low Hb A₂ due to iron deficiency, alpha-thalassaemia or delta gene defects (3). If a person is heterozygous for a δ -globin gene defect, an abnormal Hb A₂ and/or a reduction in the Hb A₂ level will be measured. It is important to identify the presence of delta gene defects, particularly during first level beta thalassaemia diagnostics (screening) for the identification of couples at risk of getting a child with a severe disease. This is because a delta defect can mask the presence of beta thalassaemia trait. The co-existence of a delta gene defect will decrease the HbA₂ level of the beta thalassaemia carrier to a normal range, and microcytosis could be attributed to alpha thalassaemia which is very frequent in many countries and particularly in Oman (6). This could compromise the basic diagnosis of beta thalassaemia trait during genetic counseling. For that, it is essential to be aware of the existence of delta gene defects for diagnostic purposes. In this study, we present the occurrence of common, rare and new delta gene mutations in a cohort of independent Omani patients.

MATERIAL AND METHODS

Out of a total of approximately 3,400 individuals, we have selected 33 independent cases attending our clinics for haemoglobinopathy screening. All cases were of Omani ethnicity. The age average was 31 and the gender was 60% females and 40% males. Samples were selected upon giving a low value of Hb A₂ (<1.9%) and/or showing second Hb A₂ fractions. Measurements were done using High Performance Liquid Chromatography (HPLC) on the Variant II (Bio-Rad Laboratories, USA) as previously described (4). DNA was extracted from whole blood, using the Qiagen kit as per the manufacturer instructions. Polymerase Chain reaction was performed as previously reported (5). The PCR products were sequenced using an ABI Prism 3730 DNA sequencer (Applied Biosystems, Perkin Elmer Corporation, Foster City, CA, USA). Iron status was not performed in all samples as it is not a mandatory test in Oman. Beta and alpha gene defects were examined at the molecular level as previously described (6).

RESULTS

Out of the 33 cases selected, 20 were found to either carry a known and/or a novel delta-globin gene mutation revealing a frequency of at least 60% in the selected group and of at least 0.6% in the random population. Eight different known mutations were observed and two novel ones.



The 2 novel mutations:

Cd147 TGA>TTA

This new mutation (HBD: c.443 G>T) resides in the stop codon of the delta gene and was found in one patient with 1.8% Hb A₂ (Figure 8.1a). The mutation results in an elongation of the transcript with 15 extra amino acids before reaching the new stop codon (TAG).

Cd110-Cd111 (+GT)

Another new delta-thalassemia mutation (HBD c.333-334 insGT) was found in a patient with 1.5% Hb A₂. The mutation involves an insertion of 2 nucleotides (+GT) between codon 110 and codon 111 (Figure 8.1b). The outcome of this insertion is a frameshift with a new stop codon (TAG), 102 amino acids beyond the insertion site. All data are summarized in Table 8.1.

Table 8.1. Summary of the delta-globin gene mutations found in 20 cases. Mutations marked with * are novel.

Sample #	HbA ₂	HbX	HBD mutation	HBD HUGO nomenclature	α -genotype	Other mutations
1	2.1	1.5	Cd16 GGC>CGC	c.49G>C	$\alpha\alpha/\alpha\alpha$	
2	1.3	1.2	Cd16 GGC>CGC	c.49G>C	$-\alpha/-\alpha$	
3	1.5	1.4	Cd16 GGC>CGC	c.49G>C	$-\alpha/\alpha\alpha$	
4	1.7	1.1	Cd16 GGC>CGC	c.49G>C	$\alpha\alpha/\alpha\alpha$	
5	1.5	1.0	Cd16 GGC>CGC	c.49G>C	$-\alpha/-\alpha$	
6	1.3		Cd116 CGC>CAC	c.350G>A	$-\alpha/\alpha\alpha$	
7	1.6		Cd116 CGC>CAC	c.350G>A	$\alpha\alpha/\alpha\alpha$	
8	1.4		Cd116 CGC>CAC	c.350G>A	$-\alpha/\alpha\alpha$	
9	1.7		Cd27 GCC>TCC	c.82G>T	$-\alpha/-\alpha$	
10	1.6		Cd27 GCC>TCC	c.82G>T	$-\alpha/\alpha\alpha$	
11	0.6		Cd27 GCC>TCC/ IVS-1-128 G>C	c.82G>T/c.93-1 G>C	$-\alpha/\alpha\alpha$	
12	1.2		Cd136 GGT>GAT	c.410G>A	$-\alpha/-\alpha$	
13	1.3		Cd136 GGT>GAT	c.410G>A	$-\alpha/-\alpha$	
14	1.9		- 68 C>T	c.-118C>T	$-\alpha/\alpha\alpha$	
15	5.1	1.7	- 68 C>T	c.-118C>T	$-\alpha/-\alpha$	HBB:c.20A>T/ c.92+5G>C
16	1.3		Cd4 ACT>ATT	c.14C>T	$-\alpha/\alpha\alpha$	
17	1.6		IVS-1-128 G>C	c.93-1 G>C	$-\alpha/\alpha\alpha$	
18	4.4		Cd100 CCT>TCT	c.301 C>T	$-\alpha/-\alpha$	HBB:c.+108_ +112delAATAA
19	1.8		Cd147 TGA>TTA*	c.443 G>T	$-\alpha/-\alpha$	
20	1.5		Cd110-Cd111 (+GT)*	c.333-334 insGT	$-\alpha/\alpha\alpha$	

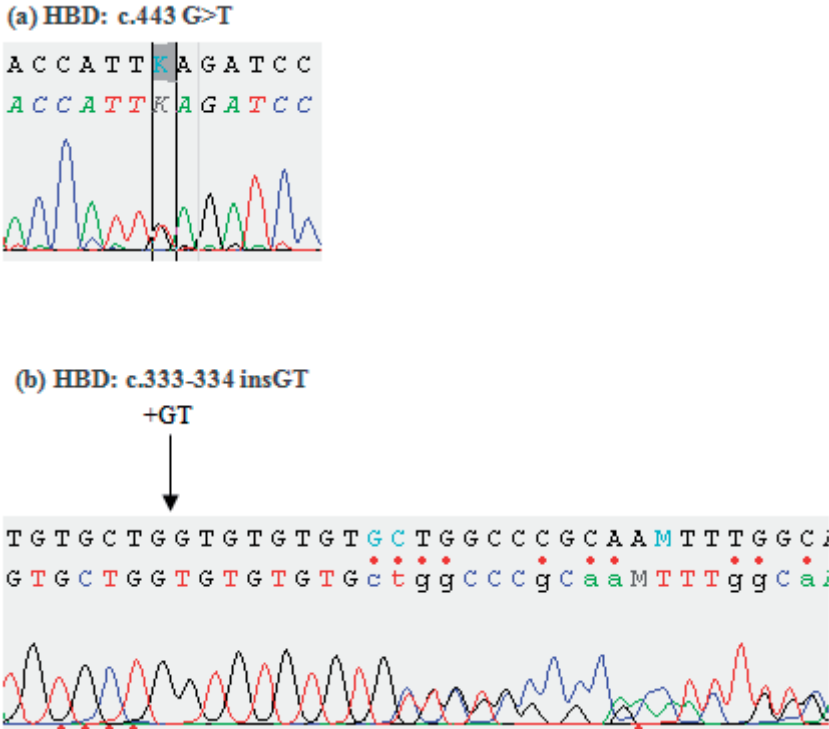


Figure 8.1. Sequence results of the 2 new delta-globin mutations found in this study.

Out of these 33 cases, 16 had the $(-\alpha/-\alpha)$ genotype, 13 had the $(-\alpha/\alpha\alpha)$ genotype and only 4 cases were normal for the alpha genes. In 13 cases, no association was found between low Hb A_2 levels and delta-globin mutations.

DISCUSSION

Delta-gene defect were found in 73.3% of the cases with low or abnormal Hb A_2 separation while in 13 out of 33 individuals, no association was found between the low Hb A_2 levels and mutations in the delta-globin gene. Iron deficiency and/or alpha thalassemia can cause a reduction in the normal Hb A_2 level while lower amount of total hemoglobin loaded on the HPLC column can also be accounted for artifacts (3).

Hb A₂' or HbB₂

Hb A₂' is stable and produces a second Hb A_2 peak and is mainly found in Africans (7). This mutation could have arrived to Oman by gene flow due to the past trading contact between Oman and Zanzibar. In one case, this delta variant was linked to codon 97 (HBD:c.294C>T) with a neutral change of the amino acids (His>His). This neutral polymorphism was previously described in Greek Cypriots (8).

Hb A₂ – Coburg

The Hb A₂ Coburg peak cannot be detected on HPLC because it co-migrates in the tail of HbA (9). This variant has been described in Sicilian families in trans to a beta thalassemia allele, reducing the Hb A₂ level to normal (10). Due to the Arab domination in Sicily, the mutation could be of African origin.

Hb A₂ – Yialousa

Hb A₂-Yialousa is the most frequent delta-globin mutation in the Mediterranean area, probably indicating a common South European origin (11). Being Hb A₂-Yialousa one of the common delta defects found in the Portuguese (12), the presence of the mutation in Omanies could be associated with the history of the Portuguese domination in Oman (1507 – 1650) (13).

Hb A₂ – Babinga

HbA₂-Babinga was primarily described in Babinga pygmies living in the Central African Republic and other African populations (14) and it could be compatible with the African ancestry of Omani tribes. A homology of this defect was also found in the beta globin gene (Hb Hope, β 136 Gly>Asp) (15). We have found this mutation in 2 individuals from the northern part of the country. These patients were anemic, presenting with low Hb A₂ levels (1.2 and 1.3% respectively) and had a normal iron profile.

5'UTR (- 68 C>T)

The delta-thalassemia promoter defect (HBD c.-118C>T, -68 C>T), has no homology to the β -globin gene (5) but the CCAAT sequence residing in the β -globin gene promoter is considered to be a regulatory element, critical for the correct initiation and high level of transcription in the globin genes (16). Therefore, the δ (CCAAC to CCAAT) mutation can be considered responsible for the lower transcription level of the δ -globin gene.

Cd4 ACT>ATT

The nucleotide change C to T at the second position of codon 4 resulting in a Thr > Ile single amino acid substitution was first described in a Greek patient (8). The variant is unstable and behaves as a thalassaemic defect with a low Hb A₂ value (1.3%) slightly lower than what was found in the Greek patient (1.4%) (8), possibly due to the coexisting heterozygous $-\alpha^{3,7}$ deletion in our patient.

(δ°): IVS-I-128 G>C

A 59-year-old male from Muscat, showed a very low Hb A₂ value (0.6%). Sequencing of the δ -globin gene revealed compound heterozygosis for two different mutations: The known A₂ Yialousa (HBD c.82G>T) and IVS-I-128 G>C (HBD: c.93-1 G>C). We believe that the IVS-I-128 mutation reduces or nearly abolish the efficiency of the 3' splicing site, leading to a deficiency in mRNA production. Compound heterozygosis for delta globin gene defects with very low Hb A₂ values observed in our patient have been reported in few cases. Amirian et al. reported a patient from Iran with two delta defects (HBD:c.92+5G>T and c.428C>A) with 0.6% Hb A₂ (17). We found the δ° IVS-I-128 mutation also solely in another patient with Hb A₂ value of 1.6%.

(δ^+):Cd100 CCT>TCT

We have observed this recently reported delta variant (HBD c.301 C>T) with a Serine substituting a Proline in a single patient who was also a carriers of a beta thalassemia mutation (HBB:c.110_114del) This delta mutation was recently published by Colaco et al. as Hb A₂-Saurashtra (20) and was found in cis with (HBB:c.110_114del). This is the same beta-thalassemia mutation found in the present paper, indicating that the HBD:c.301C>T and HBB:c.110_114del mutations may also be in cis.

(δ°): Cd147 TGA>TTA

This novel δ -stop codon mutation (Cd147 TGA>TTA) results in an elongation of the transcript with 15 additional amino acids, stopping at the 16th codon (TAG). . The elongated chain is unsuitable for functional tetramer formation and is probably proteolysed.

(δ°): Cd110-Cd111 (+GT)

Finally, the last sample showed a novel insertion of two nucleotides between codon 110 and codon 111 in exon 3 of the delta globin gene. The frame shift results in an elongated sequence with a new stop codon (TAG) 102 amino acids further from the insertion site. This mutation could also be the result of a duplication event as the region is characterized by a nucleotide repeat of (GTGTGTGT).

CONCLUSIONS

We have shown that lower Hb A₂ levels are often associated with δ -globin gene defects that may compromise screening for β -thalassemia trait when the diagnosis is based on the Hb A₂ level solely. Moreover, low levels of Hb A₂ can also be due to iron deficiency and/or alpha thalassemia due to preferential binding of the erythroid elements (3). The latter was observed in the 13 samples that had a normal delta- gene sequence. Hb A₂ levels can be moderately lowered in patient with iron deficiency due to the preferential binding of the heme to the beta and alpha chains rather than to delta chains (18).

Our results show that δ gene mutations are present in Oman at a considerable frequency and that attention should be paid during haemoglobinopathy screening to not miss beta thalassemia carriers. Double Hb A₂ fractions must be summed up to calculate the real Hb A₂ level. Samples with low Hb A₂ and microcytosis should always be checked for iron depletion before checking the alpha genotype and the δ and β globin genes sequences. Eventually, loading a more concentrated sample on HPLC is advisable when an unstable δ -globin gene variant is suspected (19). It should also be noted that in $\delta\beta$ -thalassemia deletions, the level of Hb F is usually raised while the level of Hb A₂ will remain normal. Only in solely δ -thalassemia cases, the Hb F level will stay normal (17).

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The authors declare to have conducted this study according to local ethical regulations and to have no conflicts of interest on the presented matters.



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