CHAPTER 7

MOLECULAR SPECTRUM OF α-GLOBIN GENES DEFECTS IN OMANI

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ABSTRACT

We describe the molecular characterization of α-globin gene defects in a cohort of 634 Omani patients. A total of 21 different α-gene mutations were found in 484 subjects. Overall, we identified 3 different large deletions, 3 small deletions, 11 point mutations (2 in the poly A tail of α_2 and 9 alpha-chain variants), 3 $\alpha\alpha\alpha^{anti\ 3.7}$ triplication, a 21nt duplication in the α_1 gene and 2 novel presumed polymorphisms in the alpha 3.7kbp hybrid gene namely; -5 C>T and + 46 C>A. Out of these defects, 15 have not been previously reported in the Omani population. This large heterogeneity of α-thalassemia observed in the Omani population could be expected in neighbouring Arab countries. The high frequency of α-thalassemia, solely or in association with β-globin gene defects, emphasize the necessity of adding α-thalassemia testing to pre-marital programs for accurate genetic counselling.

INTRODUCTION

Alpha-thalassemia, one of the commonest autosomal recessive diseases in man, results from the absence of expression of one or more of the four α-globin genes and can result in phenotypes ranging from asymptomatic to severe or lethal hemolytic anemia (1). The majority of the mutations causing α-thalassemia are deletions involving one or both α-genes on chromosome 16, leading to α^+ or α^0 defects respectively, being the so called rightward ($-\alpha^{3.7}$kb) deletion the most common worldwide (1). However, a growing number of non-deletion defects have been identified, being the α_2 polyadenylation signal mutation (HBA2:c.*94A>G: AATAAA>AATAAG) and the α_2 IVS-I 5-bp deletion (HBA2:c.95+2_95+6delTGAGG) the most common. These two mutations have already been reported in a study conducted in Oman, aimed to investigate the spectrum of α- thal mutations in HbH patients and in newborns showing the Hb-Bart's fraction (2). However, no studies have been conducted on Omanis to determine the prevalence of α-thal mutations in β-hemoglobinopathy patients as well as in individuals with microcytic hypochromic red cell indices (low MCV and low MCH). The present work gives a more extended picture of the prevalence and spectrum of α-gene mutations in Omanis. Characterization of co-existing α-globin gene defects is essential in prevention programs as well as in tailoring treatment strategy.

MATERIALS AND METHODS

Between 2010 and 2013, a cohort of 634 individuals was selected attending Ministry of Health Hospitals in Oman for hemoglobinopathy screening. Consent form was obtained from all patients through their direct clinicians. The median age was 22 years and the gender ratio was 58% males and 42% females. The cohort was subdivided into 7 categories (Table 7.1) based on their clinical and/or haematological profiles. Group 1 consisted of 487 patients with either β-thalassemia major, β-thalassemia minor, sickle cell trait or sickle cell disease. Group 2 counted 93 subjects with normal HPLC readings and hypochromic microcytic red cell indices. Group 3 consisted of 32 patients with normal HPLC and normal red cell indices. Group 4 consisted of 11 patients with un-known peaks on HPLC and normal β-globin gene sequence. Group 5 of 5 patients with un-explained anemia. Group 6 of 4 HbH patients and finally group 7 of only two patients with low HbA_2, normal δ-globin gene sequence and normal Ferritin values.

Blood samples were collected in EDTA, had complete blood count (CBC) and were analyzed on High Performance Liquid Chromatography (HPLC) using the Variant II (Bio-Rad Laboratories, USA) as previously described (3). Genomic DNA was extracted from whole blood, using the Qiagen kit as per the manufacturer instructions. Alpha-globin genotype of the 634 cases was established by gap-PCR for the most common 7 alpha thalassemia deletion defects (4). Groups 4-7 underwent direct sequencing of the α_2- and α_1-globin genes using an ABI Prism 3730 DNA sequencer (Applied Biosystems, Perkin Elmer Corporation, Foster City, CA, USA) as previously described (5). Groups 1 and 2 were sequenced following specific criteria (Figure 7.1).

Table 7.1. Subdivision of the 634 subjects based on their clinical and hematological data.

Group	no. of patients	category
1	487	β - thalassemia minor/major, SCT or SCD
2	93	Normal HPLC but with mycrocitic, hypochromic (MCV < 78fl; MCG < 26pg)
3	32	Normal HPLC, normal red cell indices (MCV > 78fl; MCH >26pg)
4	11	Un-known fraction on HPLC (Hb X) with a normal β - gene sequence
5	5	Un-explained anaemia, normal HPLC ,mycrocitic, hypochromic, not responding to iron
6	4	HbH peak on HPLC, anemic, mycrocitic, hypochromic
7	2	Low HbA_2 , normal δ - gene sequence, normal iron but microcitic, hypochromic
Total	634	

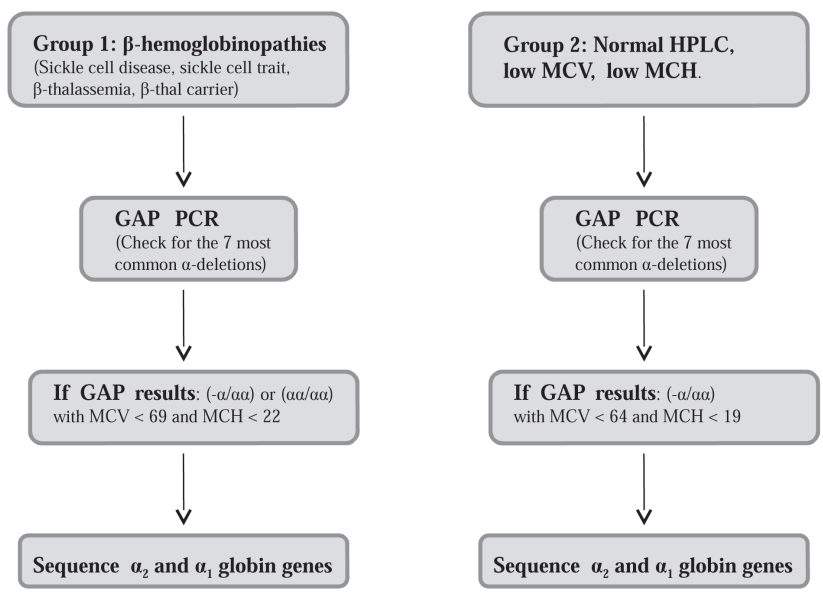

Figure 7.1. Criteria used to sequence α_2- and α_1-globin genes in groups 1 and 2.

RESULTS

In addition to the expected high prevalence of deletions such as ($-\alpha^{3.7}$) and ($-\alpha^{4.2}$) (6), we found in group 1 an un-expected number of α-mutations interacting with β-defects. A total of 10 defective alleles were found in this group. Three have been previously reported in the Omani population; the common (-5nt) deletion at the splice donor site of the α_2-globin gene, the α_2 cd19 (-G) (HBA2:c.56delG) mutation and the α_2 polyadenylation signal mutation +94 A>G. The other 7, are reported for the first time in the Omani population; the $\alpha\alpha\alpha^{anti\ 3.7}$ triplication,

α_1 21nt duplication (+GACCCGGTCAACTTCAAGG TG) between IVS-II-3 and IVS-II-4 in a beta-thalassemia carrier patient (MCV=75.3fl, MCH=18.9pg), the α_2 polyadenylation signal mutation (HBA2:c.*92A>G), Hb Le Lamentine Cd20 CAC>CAA (HBA2:c.63C>A) in HbS carrier with HbX=25.8%, Hb Tatras Cd7 AAG>AAC (HBA2:c.24G>C) in another HbS carrier with HbX=12.7%, Hb Al Ain Cd18 GGC>GAC (HBA2:c.56G>A) in a third HbS carrier with HbX=1.5%, and finally a novel mutation (+46 C>A) in the poly A tail of the alpha2/alpha1 ($-\alpha^{3.7}$) hybrid gene. The haematology of the latter mutation is summarized later in Table 4 as the same allele was also found among patients from group 5.

Moreover, among group 1, 78 individuals were carriers of HbS (SCT). Thirty were homozygous (-α/-α) (38.5%), 24 heterozygous (-α/αα) (30.8%), 3 heterozygous for α-chain variants mentioned above (3.8%) and 21 normal (αα/αα) (26.9%). HbS levels in SCT were inversely proportional to the associated number of alpha-thalassemia genes. Average levels were as follows; -α/-α (HbS % »26.6), -α/αα (HbS% » 32.5), αα/αα (HbS % »37.7) (Table 7.2).

Table 7.2. The effects of α-thalassemia on the average HbS% in Omani SCT individuals.

	Hb S (%)	MCV (fl)	MCH (pg)
(-α/-α) n=30	26.6	62.8	20.9
(-α/αα) n=24	32.5	72	23.9
(αα/αα) n=21	37.7	79	26.4

In group 2, besides high percentages of ($-\alpha^{3.7}$) and ($-\alpha^{4.2}$) deletions, we found a 12 year old female who presented with a normal clinical picture with low MCV = 64.2fl and MCH = 19pg, and was found to be heterozygous for the $-\alpha^{3.7}$ deletion and hemizygous for the α_2 Hb Dagestan variant Cd 60 AAG>GAG (HBA2:c.181A>G). This electrophoretically I-like variant is not associated with any clinical symptoms or haemoglobin instability in the carrier state.

In group 3, although patients had a normal haematological data, 43.75% and 6.25% of the patients were carrier of the ($-\alpha^{3.7}$) and ($-\alpha^{4.2}$) deletions respectively.

In group 4, we report the identification of 6 alpha-variant alleles that are reported for the first time in Omani. Two of these variants have been also observed in two HbS carriers from group 1. The variants detected were; the α_2 Hb Fontainebleau Cd21 GCT>CCT (HBA2:c.64G>C), α_2 Hb-Tatras Cd7 AAG>AAC, α_2 Hb Al-Ain Abu Dhabi Cd18 GGC>GAC, α_1 Hb Evanston Cd14 TGG>CGG (HBA1:c.43T>C), α_2 Hb Constant Spring Cd142 TAA>CAA (HBA2:c.427T>C) and the α_2 Hb J-Paris-I Cd12 GCC>GAC (HBA2:c.38C>A) (Table 7.3).

We found a new single nucleotide substitution in the 3' PolyA tail (+46 C>A) on the (α-3.7) alpha2 alpha1 hybrid gene in homozygous state ($-\alpha^{3.7\ (+46\ C>A)}$/ $-\alpha^{3.7\ (+46\ C>A)}$) in 4 anaemic patients from group 5. This allele was also observed in heterozygous state in 3 patients from group 1 ($-\alpha^{3.7\ (+46\ C>A)}$/αα). Among group 5, two patients had borderline ferritin values and were treated with iron without improvement (Table 7.4) suggesting that 3'(+46 C>A) might be a new candidate

Table 7.3. Hematological data of group 4 with Hb alpha chain variants.

Genotype	HGVS nomenclature	patient no.	HbX %	MCV (fl)	MCH (pg)
1. $\alpha_2^{Cd21\ GCT>CCT}\alpha_1/\alpha\alpha$	HBA2:c.64G>C	1.1	8.7	60.2	18.7
		1.2	5.8	82.2	26.5
		1.3	11.6	69.7	20.7
2. $-\alpha^{3.7}/\ \alpha_2^{Cd21\ GCT>CCT}\ \alpha_1$	HBA2:c.64G>C	2.1	9.8	57.2	16.5
		2.2	12.2	75	22.1
3. $\alpha_2^{Cd7\ AAG>AAC}\alpha_1/\alpha\alpha$	HBA2:c.24G>C	3.1	19.7	70.6	24.2
		3.2	12.7	73.3	24.2
4. $-\alpha^{3.7}/\ \alpha_2^{Cd18\ GGC>GAC}\ \alpha_1$	HBA2:c.56G>A	4.1	22.2	78.4	24.2
		4.2	30.5	78.5	25.3
5. $\alpha_2^{Cd18\ GGC>GAC}\ \alpha_1/\alpha\alpha$	HBA2:c.56G>A	5.1	12.9	88.9	28.7
6. $\alpha_2\alpha_1^{Cd14\ TGG>CGG}/\ \alpha\alpha$	HBA1:c.43T>C	6.1	0.6	77.9	23.6
7. $\alpha_2^{Cd142\ TAA>CAA}\ \alpha_1/\ \alpha_2^{Cd142\ TAA>CAA}\ \alpha_1$	HBA2:c.427T>C	7.1	4.6	78.3	22.4
8. $\alpha_2^{Cd12\ GCC>GAC}\ \alpha_1/\ \alpha_2^{-5nt}\ \alpha_1$	HBA2:c.38C>A/ HBA2:c.95+2_95+6delTGAGG	8.1	36.7	82.9	25.2

Table 7.4. Hematological findings of the 3' PolyA tail (+46 C>A) cases found in the alpha2/alpha1 3.7 hybrid gene. (HR) = Heterozygous.

alpha - genotype	patient no.	beta - genotype	Age	HbA_2 %	Hb (g/dl)	MCV (fl)	MCH (pg)	Ferritin	Phenotype
1. $-\alpha^{3.7\ (+46C>A)}/\ \alpha\alpha$	1.1	HBB:c.92+5G >C (HR)	2y	4.3	8.8	55.1	16.5	normal	mild anemia
	1.2	HBB:c.92+5G >C (HR)	1y	5.9	9.2	56.5	18.2	normal	mild anemia
	1.3	HBB:c.93-21_ 96del (HR)	7y	6.1	10.4	57.6	18.1	not done	mild anemia
2. $-\alpha^{3.7\ (+46\ C>A)}/\ -\alpha^{3.7\ (+46\ C>A)}$	2.1	normal	1y	3.5	8	76.1	23.4	not done	anemia and jaundice
	2.2	normal	15y	1.8	9.2	57.6	18.7	normal	anemia
	2.3	normal	39y	2.6	12.5	65.9	21	border line	anaemia not responding to Fe therapy
	2.4	normal	5y	2.3	7.6	47.6	13.6	border line	anaemia not responding to Fe therapy
3. $-\alpha^{3.7\ (-5\ C>T)}/\ -\alpha^{3.7}$	3.1	normal	56y	1.3	8.2	64.5	20.9	normal	hypochromic microcytic anemia

mutation. In addition, among group 5, we identified the -$\alpha^{3.7}$/ $\alpha_2\alpha_1^{Cd38/39(-ACC)}$ genotype in a patient with a mild anaemia (Hb=6.8, MCV=66.8 and MCH=22.5). We believe that this is the first study reporting the α_1 Hb Taybe Cd38/39 –ACC (HBA1:c.118_120delACC) deletion allele in Omani.

In group 6, 4 HbH genotypes were identified, three genotypes in a combination that has been previously reported in the Omani and one with the $-\alpha^{4.2}$/ $\alpha_2^{+94\ A>G}\alpha_1$ genotype which is observed for the first time in a patient from northern Oman (Khasab).

Among group 7, we characterized two single nucleotide substitutions, one in the 5' promoter (-5 C>T) in the alpha2/alpha1 hybrid gene which was found in heterozygous form in a patient with homozygous α-3.7kbp deletion (-$\alpha^{3.7\ (-5\ C>T)}$/-$\alpha^{3.7}$). The patient presented with hypochromic microcytic anaemia (MCV=64.6fl, MCH=20.9pg), low HbA_2 (1.3%) and normal ferritin value. The second, in a patient homozygous for Hb Icaria variant Cd142 TAA>AAA (HBA2:c.427T>A) with low HbA_2 (1.6%) and microcytic hypochromic (MCV=76.3fl, MCH=24pg). The first mutation could be a novel thalassemia defect down regulating the hybrid gene and the second is described for the first time in Oman.

Mutations found in all groups are summarized in Table 7.5.

Table 7.5. Summary of the spectrum of alpha-globin genotypes found in our cohort subdivided into 7 groups. (alleles marked with * might be new candidates).

	Genotype Mutation	HGVS allele nomenclature	no. of patients	genotype prevalence among the sub-group (%)
Group 1 (n = 487)	-$\alpha^{3.7}$/-$\alpha^{3.7}$		171	35.1
β-hemoglobinopathies	-$\alpha^{3.7}$/-αα		145	29.8
	-$\alpha^{3.7}$/-$\alpha^{4.2}$		10	2.1
	-$\alpha^{4.2}$/-αα		10	2.1
	ααα$^{anti\ 3.7}$ /αα		3	0.6
	-$\alpha^{3.7(+46\ C>A)*}$/ αα		3	0.6
	$\alpha_2^{-5nt}\alpha_1$/ αα	HBA2:c.95+2_95 +6delTGAGG	2	0.4
	$\alpha_2^{+92\ A>G}\alpha_1/\alpha_2^{+92\ A>G}\alpha_1$	HBA2:c.*92A>G	1	0.2
	$\alpha_2^{+92\ A>G}\alpha_1$/αα	HBA2:c.*92A>G	1	0.2
	$\alpha_2^{Cd19(-G)}\alpha_1$/αα	HBA2:c.56delG	1	0.2
	$\alpha_2^{Cd19(-G)}\alpha_1/\alpha_2^{Cd19(-G)}\alpha_1$	HBA2:c.56delG	1	0.2
	$\alpha_2^{+94\ A>G}\alpha_1$/ αα	HBA2:c.*94A>G	1	0.2
	$\alpha_2\alpha_1^{dup\ 21nt}$ / αα	HBA1:c.283_300+3dup	1	0.2
	-$\alpha^{3.7}/\alpha_2^{Cd20\ CAC>CAA}\alpha_1$	HBA2:c.63C>A	1	0.2
	$\alpha_2^{Cd7\ AAG>AAC}\alpha_1$/αα	HBA2:c.24G>C	1	0.2
	$\alpha_2^{Cd18\ GGC>GAC}\alpha_1$/αα	HBA2:c.56G>A	1	0.2
	-$\alpha^{4.2}/\alpha_2^{+92\ A>G}\alpha_1$	HBA2:c.*92A>G	1	0.2
	αα/αα		133	27.3

Table 7.5. Summary of the spectrum of alpha-globin genotypes found in our cohort subdivided into 7 groups. (alleles marked with * might be new candidates). (*Continued*)

	Genotype Mutation	HGVS allele nomenclature	no. of patients	genotype prevalence among the sub-group (%)
Group 2 (n = 93)	$-\alpha^{3.7}/-\alpha^{3.7}$		72	77.4
Normal HPLC,	$-\alpha^{3.7}/-\alpha\alpha$		17	18.3
hypochromic, mycrocytic	$-\alpha^{3.7}/-\alpha^{4.2}$		3	3.2
red cell indices	$-\alpha^{3.7}/\alpha_2^{Cd60\ AAG>GAG}\alpha_1$	HBA2:c.181A>G	1	1.1
Group 3 (n = 32)	$-\alpha^{3.7}/-\alpha\alpha$		14	43.75
Normal HPLC,	$-\alpha^{4.2}/-\alpha\alpha$		2	6.25
Normal red cell indices	$\alpha\alpha/\alpha\alpha$		16	50
Group 4 (n = 11)	$\alpha_2^{Cd21\ GCT>CCT}\alpha_1/\alpha\alpha$	HBA2:c.64G>C	3	27.2
Un-known peak on HPLC	$-\alpha^{3.7}/\alpha_2^{Cd21\ GCT>CCT}\alpha_1$	HBA2:c.64G>C	2	18.2
Normal β-globin gene seq	$-\alpha^{3.7}/\alpha_2^{Cd18\ GGC>GAC}\alpha_1$	HBA2:c.56G>A	2	18.2
	$\alpha_2^{Cd7\ AAG>AAC}\alpha_1/\alpha\alpha$	HBA2:c.24G>C	1	9.1
	$\alpha_2\alpha_1^{Cd14\ TGG>CGG}/\alpha\alpha$	HBA1:c.43T>C	1	9.1
	$\alpha_2^{Cd142\ TAA>CAA}\alpha_1/\alpha_2^{Cd142\ TAA>CAA}\alpha_1$	HBA2:c.427T>C	1	9.1
	$\alpha_2^{Cd12\ GCC>GAC}\alpha_1/\alpha_2^{-5nt}\alpha_1$	HBA2:c.38C>A/ HBA2:c.95 +2_95+6delTGAGG	1	9.1
Group 5 (n = 5)	$-\alpha^{3.7(+46\ C>A)*}/-\alpha^{3.7\ (+46\ C>A)*}$		4	80
un-explained anaemia	$-\alpha^{3.7}/\alpha_2\alpha_1^{Cd38/39\ (-ACC)}$	HBA1:c.118_120delACC	1	20
Group 6 (n = 4)	$\alpha_2^{+94\ A>G}\alpha_1/\alpha_2^{+94\ A>G}\alpha_1$	HBA2:c.*94A>G	1	25
HbH disease	$-\alpha^{4.2}/\alpha_2^{+94\ A>G}\alpha_1$	HBA2:c.*94A>G	1	25
	$-\alpha^{3.7}/\alpha_2^{+94\ A>G}\alpha_1$	HBA2:c.*94A>G	1	25
	$-\alpha^{3.7}/-\alpha^{MedI}$		1	25
Group 7 (n =2)	$-\alpha^{3.7\ (-5\ C>T)*}/-\alpha^{3.7}$		1	50
low HbA_2, normal δ and Fe	$\alpha_2^{Cd142\ TAA>AAA}\alpha_1/\alpha_2^{Cd142\ TAA>AAA}\alpha_1$	HBA2:c.427T>A	1	50

DISCUSSION

The broad range of α-thalassemia defects identified in the current study, demonstrate that the heterogenic pattern prevalent in Oman is similar to that reported in Saudi (7). This can be accounted to gene-flow within the nomadic populations of the Arab peninsula and to the past trade with other countries as well as to the past Portuguese domination in Oman. A total of 21 different α-gene defects have been reported in this study. Six defects have been previously identified in the Omani population (2,6,8). These include the most common $\alpha^{3.7}$, $\alpha^{4.2}$ and $--^{MEDI}$ large deletions, but also the less common Mediterranean donor site IVS-I (-5nt) deletion, the

polyadenylation signal site (+94 A>G) point mutation of the α2 globin gene affecting the RNA transcription termination (9) and the point deletion Cd19 (-G) which was first reported in the Iranian population, resulting in premature termination of the α-globin chain (5).

The majority of our cohort carried the large $\alpha^{3.7}$ deletion either in homozygous or heterozygous states followed by $\alpha^{4.2}$ and a minority consisted of α-small deletions or point mutations including Hb-variants. In one patient, a 21nt duplication was identified which most probably arose as a consequence of homologous crossing over between the two alpha-1 globin genes. Although the original splice donor site remains intact but having two sites might result in the mutant allele using most times the original one and sometimes the other one resulted from the duplication which is located in the intron creating an instable mRNA due to nonsense mediated decay. This might also explain why having this mutation appears very mild.

Among group 1, an important remark is made with respect to genetic counselling on one hand and the alpha spectrum on the other hand is when the group of beta-thalassemia carriers or sickle cell trait couples are counselled for a beta-thalassemia major or sickle cell disease in the offspring overlooking an important alpha-thalassemia mutation that can result in a severe HbH disease in the offspring in combination with an alpha zero-thalassemia carrier that can go 'unnoticed' by only looking at the beta-thalassemia parameters and not to the alpha-thalassemia mutation spectrum.

Alkindi et al studied 32 HbH Omani patients and found that the most common α-globin genotype was $\alpha_2^{+94\ A>G}\ \alpha_1/\ \alpha_2^{+94\ A>G}\ \alpha_1$ followed by $-\alpha^{3.7}/--^{MED}$ (2). We found the same two genotypes in group 6 (patients with HbH) along with another two genotype combinations; $-\alpha^{4.2}/\ \alpha_2^{+94\ A>G}\alpha_1$ and $-\alpha^{3.7}/\ \alpha_2^{+94\ A>G}\alpha_1$. The latter has been previously reported in an Omani family study (8).

It is not clear to which extent the homozygous mutation $-\alpha^{3.7(+46\ C>A)}/\ -\alpha^{3.7(+46\ C>A)}$ found in four patients in group 5, with haemolytic anaemia, might affect the phenotype by reducing the expression of the $-\alpha^{3.7}$ gene. If the same $-\alpha^{3.7(+46\ C>A)}$ allele reported by Alkindi's in 4 HbH patients with $-\alpha^{3.7(+46\ C>A)}/-\alpha^{3.7}$ genotype (2) would suggest a total expression failure, then our homozygous case should have been lethal hydrops foetalis, which was not the case. Hb H disease is the only intermediate form of α-thalassemia compatible with postnatal life and the clinical severity of the disease is associated with the type of mutation (1). The typical genotype of Hb H disease results from the loss of three functional genes due to large deletions. Less frequently, more severe combinations of α° deletion defects with point mutations such as Hb Constant Spring or other similar defects, while less severe HbH forms may arise from combinations with poly A signal mutations (10). However, a point mutation on the $-\alpha^{3.7}$ hybrid might down regulate or disrupt the allele expression causing severe forms. The risk of developing anaemia in case of homozygous + 46 C>A on the alpha2/alpha1 hybrid gene highlights the importance of screening for α-thalassemia defects by molecular analysis rather than by routine blood count alone which is based on detecting hypochromic microcytic red cell indices.

Although not always clear, we have provided additional evidence that besides cases with delta thalassemia or consistent iron deficiency, a reduction in HbA_2 level can be associated with α-thalassemia as shown for the novel -5C>T allele in the $-\alpha^{3.7(-5C>T)}/\ -\alpha^{3.7}$ genotype. Therefore, in patients originating from areas where haemoglobinopathies are common, it is necessary to perform molecular tests to clearly differentiate between iron deficiency and alpha- or delta-thalassemia for accurate diagnosis.

In conclusion, four α-thalassaemia categories were recognized in this cohort: the silent carrier, the alpha-thalassemia trait, the α -variant and the anaemic form of Hb H disease. Moreover, we have shown that 40% of the α-alleles were normal while 56% of the alpha thalassemia alleles studied were large α-thalassemia deletion and 4% non-large deletions/ point α-thalassemia mutations. The consistent occurrence of point mutations could represent a risk factor for severe haemolytic anaemia in combination with large deletions (11). The + 46 C>A on the alpha2/alpha1 hybrid gene might be a candidate mutation since it is associated with anaemia, however larger number of genotype/phenotype correlation studies should be carried out to confirm our findings. Identifying the α-globin gene spectrum is not only clinically important but also fundamental for a better genetic counselling.

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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.

REFERENCES

1. Hartevel CL. And Higgs DR. α-thalassemia. Orphanet J Rare Dis 2010; 5: 13.
2. Alkindi SS, AlZadjali S, Daar S, Sindhuvi E, Wali Y, Pathare AV, Venugopal S, Lapoumeroulie C, Srivastava A and Krishnamoorthy R. A stepwise α-thalassemia screening strategy in high-prevalence areas. European Journal of Haematology 2013; 91(2): 164-169.
3. van Delft P, Lenters E, Bakker-Verweij M, de Korte M, Baylan U, Harteveld CL and Giordano PC. Evaluating five dedicated automatic devices for haemoglobinopathy diagnostics in multi-ethnic populations. Int J Lab Hematol 2009; 31, 484–495.
4. Liu YT, Old JM, Miles K, Fisher CA, Weatherall DJ, Clegg JB. Rapid detection of α-thalassaemia deletions and α-globin gene triplication by multiplex polymerase chain reactions. Br J Haematol 2000; 108: 295–299.
5. Harteveld CL, Yavarian M, Zorai A, Quakkelaar ED, van Delft P, Giordano PC. Molecular spectrum of alpha-thalassemia in the Iranian population of Hormozgan: three novel point mutation defects. Am J Hematol 2003; 74: 99–103.
6. Hassan SM, Hamza N, Jaffer Al-Lawatiya F, Jaffer Mohammed A, Harteveld CL, Rajab A,Giordano PC. Extended molecular spectrum of beta- and alpha-thalassemia in Oman. Hemoglobin 2010; 34(2): 127-34.
7. Akhtar MS, Qaw F, Borgio F, Albuali W, Suliman A, Nasserullah Z, Al-Jarrash S and Ali A. Spectrum of α-thalassemia mutations in transfusion dependent β-thalassemia patients from the eastern province of Saudi Arabia. Hemoglobin 2013; 37(1): 65-73.
8. Wali Y, Al Zadjali S, Elshinawy M, Beshlawi I, Fawaz N, Al Kindi S, Rawas A, Alsinani S, Daar S and Krishnamoorthy R, Severity ranking of non-deletional alpha thalassemic alleles: insights from an Omani family study. European Journal of Haematology 2011; 86: 507-511.
9. Thein SL, Wallace RB, Pressley L, Clegg JB, Weatherall DJ, Higgs DR. The polyadenylation site mutation in the alpha-globin gene cluster. Blood 1988; 71: 313-319.
10. Fei YJ, Oner R, Bozkurt G, Gu LH, Altay C, Gurgey A, Fattoum S, Baysal E and Huisman THJ. Hb H Disease Caused by a Homozygosity for the AATAAA-»-AATAAG Mutation in the Polyadenylation Site of the a2-Globin Gene. Hematological Observations. Acta Haematol 1992; 88: 82-85.
11. Traeger-Synodinos J, Kanavakis E, Tzetis M, Kattamis A, Kattamis C. Characterization of nondeletion α-thalassemia mutations in the Greek population. Am J Hematol 1993; 44: 162–167.