CHAPTER 4

EXTENDED MOLECULAR SPECTRUM OF β- AND α- THALASSEMIA IN OMAN

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ABSTRACT

Sickle cell disease is known to be very common in the Omani population, although data are limited concerning β-thalassemia (β-thal). We report the molecular background of 87 unrelated patients from the Sultanate of Oman, diagnosed with β-thal major (β-TM), β-thal intermedia (β-TI) or minor. Diagnosis was based on clinical and hematological data and confirmed by molecular analysis. We found 11 different β-thal determinants in our cohort, which consists of subjects from different regions of Oman. Six of these mutations have not been previously reported in the Omani population. The prevalence of α-thal single gene deletions (-$\alpha^{3.7}$ and –$\alpha^{4.2}$) in the same cohort was very high (58.3%). These data will contribute to the implementation of a country-wide service for early molecular detection of hemoglobinopathies and for providing genetic counseling following premarital screening.

INTRODUCTION

Hemoglobinopathies are the most frequent autosomal recessive disease in man. The most common conditions are caused by mutations in the β- or the α-globin genes coding for the postnatally expressed Hb A subunits. Mutations may either change the primary structure of the gene product [abnormal hemoglobins (Hbs)] or impair the expression of the mutated gene (thalassemia). Expression defects are subdivided into α- and β-thalassemia (α- and β-thal), resulting from the defective synthesis of α- and β-globin chains, respectively. Carriers of β-thal (thalassemia minor) are mostly asymptomatic with mild anemia, while patients with β-thal major (β-TM) are severely affected, transfusion-dependent and in need of continuous iron chelation therapy. Patients who are carriers of α-thal (−α/αα, −α/−α and − −/αα) are mildly anemic, while patients with Hb H disease (−α/− −) present with an α-thal intermedia (α-TI) picture with enlarged spleen/liver, and in some cases, may need regular or occasional blood transfusions, whereas homozygous α^0-thal (− −/− −) is lethal. But for a successful bone marrow transplant, these diseases cannot be cured, and for most of these patients the only alternative is intensive treatment until premature death. More than 80% of the α^+-thal determinants are common deletions such as the $-\alpha^{3.7}$ and $-\alpha^{4.2}$. Conversely, more than 95% of the β-thal cases are due to point mutations, of which only a few are prevalent and population-specific, while many others are rare. Population isolates usually present with a narrow spectrum of mutations, while admixed populations are characterized by a large number of mutations (1).

After sickle cell disease (SCD), β-TM is the second most common severe hemoglobinopathy in Oman, a country with a population of 2.6 million inhabitants, of which 73% are native Omanis and 27% are foreign immigrants. The annual birth rate is ±50,000 newborn, of which 250 have β –TM and are in need of intensive therapy and the figures continue to rise every year. Although there has been a significant improvement in social conditions and medical care for these patients during the last three decades, life expectation and quality of life still remains poor with severe human suffering and patients remain in continuous need of burdening therapy provided by public health services which, being free of charge in Oman, represents a considerable share of public health expenses. Prevention being better than treatment, the aim of this study was to investigate the molecular spectrum of β-thal in order to enable the study of the geographical prevalence of the different thalassemia defects occurring in the different regions of Oman. Knowledge of this molecular spectrum is necessary for early diagnosis and prevention of morbidity and mortality of these most debilitating inherited disorders.

MATERIALS AND METHODS

The examined cohort consisted of 87 unrelated individuals of both genders previously diagnosed with β-TM, β-TI or minor or with sickle cell disease. Of these, 84 were Omani subjects and three were immigrants. Age ranged from 1 to 44 years old (1963–2006). Blood was collected in EDTA and patients gave written consent for the procedures to be performed according to the local ethical regulations. Routine analyses were performed in The Netherlands, by measurement of Hb fractions on a high performance liquid chromatography (HPLC) apparatus (Variant, Bio-Rad Laboratories, Hercules, CA, USA) and on a capillary electrophoresis (CE) machine (Capillarys,

Sebia, Paris, France), as described elsewhere (2). All patients were analyzed at the molecular level. DNA extraction was done by high salt technologies, either manual or automated, using the Puregene DNA Purification System (Gentra Systems, Minneapolis, MN, USA) (3). Mutation analyses of α-thal included multiplex polymerase chain reaction (gap-PCR) as described by Liu et al. (4). Point mutation analysis of the β-globin gene was done on a GeneAmp9700 (Applied Biosystems, Foster City, CA, USA) using the QIAGEN® Multiplex PCR kit (Cat. no. 206143; Qiagen GmbH, Hilden, Germany), as previously reported (5). DNA sequencing was done on an ABI PRISM™ 3730 Genetic Analyzer (Applied Biosystems) using ABI PRISM® Big Dye Terminators v2.0 Cycle Sequencing kit according to the manufacturer's instructions.

RESULTS

Routine analyses of samples freshly collected in Oman showed either the pattern of transfused β-thal patients, of sickle cell disease, either homozygous or in combinations Hb S [β6(A3) Glu→Val]/ β-thal, or of carriers of these traits. All patients were examined at the molecular level.

In total we examined 174 alleles and found 11 different β-thal determinants.

In the non Omani, four codon 39 (C>T) and two codons 41/42 (–TTCT) alleles were found. In the 168 alleles from Omani subjects, 83 chromosomes with β-thal point mutations were characterized with nine different "Omani" mutations.

The IVS-I-5 (G>C) was the most prevalent (73%), while Hb Monroe [IVS-I (–1) or codon 30 (G>C), β30(B12)Arg→Thr], codon 5 (–CT) and IVS-I (–25 bp) 3' end mutations were the second in frequency (4.5%) together with codon 39 found in immigrants. Codons 8/9 (+G) was third in prevalence (3.3%) and codons 41/42 fourth at 2.2% but in immigrants only. The remaining four mutations were observed at lower frequencies (1.1%).

We have observed six β-thal mutations that were not found in the previous study by Daar et al. (6) on 99 Omani patients in 1998. Conversely, we have not found nine other mutations that were observed by Daar et al. (6). The IVS-I-5 mutation was the most predominant in both studies which complement each other, providing an extended spectrum of the β-thal determinants in the Sultanate of Oman. The data are summarized in Table 4.1.

Table 4.2 shows the distribution of eight Omani β-thal mutations in four regions. The IVS-I-5 mutation was predominant in Batinah, Muscat and Dhakhiliyah (73.7, 86.6 and 83.3%, respectively), while the same mutation was absent in Musandam with Hb Monroe being the most prevalent. Although the number of alleles studied is too small to provide a precise assessment, these preliminary results seem to indicate that the distribution of the mutations is not uniform and further studies are required to confirm the data (Table 4.2).

We have examined the same 87 individuals for the presence of the common α-thal deletions by gap-PCR (4). In three cases the analysis failed due to technical reasons. In 35 patients no α-thal deletion was found. In 25 cases, the $-\alpha^{3.7}$ deletion was found in the heterozygous state (29.8%) and in 22 in the homozygous state (26.2%). One patient was heterozygous for the $-\alpha^{4.2}$ deletion (1.2%) and one patient presented with compound heterozygosity for the $-\alpha^{3.7}$ and $-\alpha^{4.2}$ deletions (1.2%). Thus, the prevalence of α-thal alleles in this cohort was 58.3%. The data are summarized in Table 4.3.

Table 4.1. Comparison of the molecular spectrum of β–thalassemia mutations in Oman with the data of Daar et al. (6). [a] : Found in immigrants only

	Present study (chromosomes)		Daar et al. (6) (chromosomes)	
Mutations	***n***	**%**	***n***	**%**
IVS-I-5, G>C	65	73.0	122	61.6
Codon 5, -CT	4	4.5	1	0.5
Codon 30, G>C, Hb Monroe	4	4.5	0	0.0
IVS-I (-25bp) 3' end	4	4.5	11	5.5
Codon 39, C>T [a]	4	4.5	2	1.0
Codons 8/9, +G	3	3.4	0	0.0
Codons 41/42, -TTCT [a]	2	2.3	0	0.0
IVS-I-1, G>A	1	1.1	2	1.0
Codon 41, -C	1	1.1	0	0.0
Codon 16, -C	1	1.1	0	0.0
Codon 44, -C	0	0.0	19	9.6
Hb Dhofar, β58(E2) Pro→Arg	0	0.0	13	6.6
619 bp deletion	0	0.0	8	4.0
IVS-II-1, G>A	0	0.0	7	3.5
Codons 36/37, -T	0	0.0	2	1.0
Codon 15, G>A	0	0.0	1	0.5
Codon 37, -G	0	0.0	1	0.5
Codon 30, G>A	0	0.0	1	0.5
IVS-I-110, G>A	0	0.0	1	0.5
Hb E, β26(B8) Glu→Lys	0	0.0	1	0.5
Unknown	0	0.0	6	3.0

DISCUSSION

We herein describe eleven different β-thalassemic mutations present in Omanis and three in immigrant subjects. The imported mutations need to be taken into consideration for two reasons: *(i)* because of their significant contribution (from India, The Philippines, Egypt and others) in the past to the present day spectrum of mutations in the Omani population; *(ii)* given the actual percentage (27%) of the immigrant community within Oman, health professionals and authorities must be aware of the issues in future planning strategies (7).

As Daar et al. (6) reported, we also found that the IVS-I-5 mutation is the most common (73%) in Oman but with uneven distribution across the region. This mutation is most prevalent in the northern regions of Oman Batinah, Muscat and Dhakhiliyah (Table 4.2). The second most common molecular defects are the Hb Monroe, codon 5 and the IVS-I (–25 bp) 3'end mutations

with a prevalence of 4.5%. These data are different from those reported by Daar et al. (6) in which Hb Monroe was not found and the codon 5 mutation was observed at a much lower frequency (0.5%). Moreover, the remaining mutations were observed at lower frequencies and six were not observed by Daar et al. (6), while nine mutations reported by these investigators were not observed by us. This is probably due to differences in the ethnic composition of the cohorts and to the relatively small number of individuals studied. Larger surveys are needed

Table 4.2. Regionwise variation in the distribution of Omani β–thalassemia mutations

Region	Omani alleles studied	Omani mutations found	Type of mutation	Regional prevalence (%)
Musandam	4	3	Codon 30, G>C, Hb Monroe	75.0
		1	Cd 8/9, +G	25.0
Batinah	19	14	IVS-I-5, G>C	73.7
		4	IVS-I (-25bp) 3' end	21.0
		1	Codon 30, G>C, Hb Monroe	5.3
Muscat	53	46	IVS-I-5, G>C	86.6
		3	Codon 5, -CT	5.6
		2	Codons 8/9, +G	3.8
		1	Codon 16, -C	1.9
		1	Codon 41, -C	1.9
Dakhiliyah	6	5	IVS-I-5, G>C	83.3
		1	IVS-I-1, G>A	16.7
Total	82	82	8	

Table 4.3. Spectrum of α-thalassemia mutations in Oman

(a) Allele frequencies

Mutation	Allele frequency (%)	Prevalence (%)
$-\alpha^{3.7}$ (rightward)	41.7	
$-\alpha^{4.2}$ (leftward)	1.2	
Total	42.9	58.3

(*b*) Genotype frequencies

Mutation	*n*	Genotype frequency (%)
Rightward homozygotes	22	26.2
Rightward heterozygotes	25	29.8
Compound rightward/leftward	1	1.2
Leftward heterozygote	1	1.2

to obtain a better picture regarding prevalence and distribution of thalassemic mutations in a multi-ethnic society such as Oman.

Geographical and cultural factors separate the tribal populations of Oman causing isolation and the high degree of consanguineous marriages. Consanguinity doubles the chance of generating couples at risk and if a partner is already a carrier, the chance that the other will also be a carrier in a first-cousins marriage will be 1:16. Consequently, unless otherwise appropriate prevention measures are taken, the number of affected births will continue to grow.

In spite of the high prevalence of the Hb S and IVS-I-5 mutations, less common defects must be taken into consideration. For instance, in the region of Musandam, separated from the rest of Oman by the United Arab Emirates, three out of the four unrelated alleles studied carried the Hb Monroe mutation. This variant is associated with a β-thal phenotype (8) and at risk for β-TM (9). Hb Monroe was also found in Batinah (4.8%), a region close to Musandam and was also observed in the United Arab Emirates (10). Apparently, Hb Monroe is prevalent in communities of the west coastal area of the Arabian Gulf peninsula, but this needs to be confirmed by a larger study.

Since our recruitment strategy was focused on β-thal, our cohort can be considered as random (not selected for α-thal). The prevalence of α^+-thal mutations, known to be high in Saudi Arabians (43.3%) (11), is found to be even higher in the Omani population (58.3%). With no less than 22 homozygotes for the $-\alpha^{3.7}$ deletion and 25 carriers out of the 84 unrelated individuals, is probably the highest frequency observed in the Arabian peninsula. On the other hand, none of the α^0 defects associated with Hb H or Hb Bart's hydrops fetalis were found. The reason for the absence of the α^0 defects is probably the high consanguinity due to negative selection of such lethal defects in the high consanguineous setting.

CONCLUSIONS

Our analysis has shown the existence of few common mutations but also the presence of a number of less common defects. Considering the fact that the Omani population is a society consisting of well defined groups (tribes), the distribution of the β-thal determinants is bound to be different in different regions. Our cohort was collected mainly from the northern part of

Oman because it has been previously reported by Daar et al. (6) that β-thal mutations are prevalent in these regions. Nevertheless, we presume that β-thal defects might be present at lower frequencies in other regions as well.

These data are important for organizing prevention and management of severe forms of hemoglobinopathies in Oman. Given their high prevalence, young Omani subjects are advised to get screened for hemoglobinopathies at a premarital stage either for their choice of a partner or for getting informed in advance of the risk and of the available options: not to have children, adoption or use of donor gametes or prenatal diagnosis.

Prenatal diagnosis is available in Islamic countries such as Egypt, Tunisia and Iran, and medical abortion is allowed in these countries when the fetus is found to be affected with a severe hemoglobinopathy, in accordance with a Fatwa permitting medical abortion prior to 3 months of conception in case of a severely affected fetus (Council of the World Islamic League, 15-22/07/1410 Hijri/10-17 February 1990). Medical abortion for a severely affected fetus

remains a matter of debate/discussion in Oman and views of prominent muftis are essential. Development of prevention, control and management programs of hemoglobinopathies in Oman should aim to reduce the number of affected births and simultaneously must offer the best quality of life by medical care to those born affected with an anomaly.

DECLARATION OF INTEREST

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

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