Toward prevention of Hemoglobinopathies in Oman

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CHAPTER 5

BROADER SPECTRUM OF β-THALASSEMIA MUTATIONS IN OMAN: REGIONAL DISTRIBUTION AND COMPARISON WITH NEIGHBOURING COUNTRIES

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ABSTRACT

The objective of this study was to expand and study the molecular spectrum of β-thalassemia mutations in Oman by examining cases from 7 different regions and comparing the prevalence with neighbouring countries. A total of 446 cases of β-hemoglobinoapthy were obtained and analyzed to determine the frequency and distribution of the different β-alleles. The molecular spectrum of β-thalassemia in Oman revealed the presence of 32 different mutations of different origin and 11 alleles are reported for the first time in the Omani population. The wide heterogeneous spectrum of β-thalassemia mutations found can be associated with the history of trade and migration as well as the past domination from other countries. The presented data will facilitate the development of a comprehensive prevention strategy in Oman.

INTRODUCTION

Oman is a country at the south east corner of the Arabian Peninsula. It faces the northern east part of the Arabian Gulf, bordering United Arab Emirates and Saudi Arabia in the west, Yemen in the south and is separated from Iran by a narrow sea strait from the north east. β-thalassemia is an autosomal recessive hemoglobin disorder and one of the most common genetic disease in man. Carriers of the disease are found at high frequencies in the tropic and sub-tropic regions including Oman. Untreated patients with β- thalassemia major are likely to die early in infancy and burdening treatment is needed to delay premature death. Prevention options such as premarital screening and genetic counselling of couples at risk are offered at the national level in Oman. However, this is limited to adapting partner choice as prenatal diagnosis (PD) is not offered in the country. As termination of pregnancy is not permitted in Oman, couples at risk that marry, may either accept the risk or decide to seek for PD or pre-implantation genetic diagnosis (PGD) abroad. As for PD and PGD molecular analysis is needed, knowledge of the local spectrum of mutations is essential in order to have a reliable diagnosis. Previous studies have reported a provisional spectrum of the β thalassemia alleles in Oman (1, 2). We report here the broadest spectrum of β-thalassemia mutations found in the country by examining the largest cohort studied thus far.

MATERIALS AND METHODS

Our cohort consisted of 446 unrelated individuals of native Omani nationality attending the Ministry of Health Hospitals in the country. The subjects were either affected with β-thalassemia major (TM), or HbS/β-thal or carriers of β-thalassemia. Age ranged from 1 to 48 years; 49.3% were females and 51.7% males. Patients'/parents' consent form was obtained prior to testing. The provisional diagnoses were confirmed by complete blood counts (CBC) performed on a Cell Dyn 4000 automated blood cell counter (Abbott Diagnostics, Santa Clara, CA, USA) and by biochemical analysis including high performance liquid chromatography (HPLC) (Variant, Bio-Rad Laboratories, Hercules, CA, USA). Finally DNA was extracted using the QIAamp DNA Blood Mini Kit (Qiagen Inc., Valencia, CA, USA) and the β-globin gene was amplified and sequenced on an ABI PRISM™ 3730 Genetic Analyzer (Applied Biosystems).

RESULTS

Among the 446 cases (892 chromosomes), 273 were beta-thalassemia carriers, 102 sickle/β-thalassemia compound heterozygotes and 71 had TM. In total 32 different β-thalassemia mutations were identified among 517 mutated alleles. Eleven of the β-thal alleles are reported for the first time in Oman. These include the IVS-I-128 T>G, Hb La Desirade Cd129 TGC>TGT, the promoter mutations -88 C>A and -101 C>T, IVS-II-849A>G, Cd8 (-AA), the 5nt poly A deletion 3'(+108-3'(+112), IVS-I-6 T>C, Hb Iraq Halabja Cd10 GCC>GTC, the Cd22 GAA>TAA and the PolyA 3'(+113)A>G mutation (Table 5.1).

As expected from our previous data, the IVS-I-5 G>C was still the most frequent mutation in each region except for Dhofar. The IVS-I-5 G>C allele frequency was 43.6%, while 30 different mutations accounted for the remaining 56.4%. The Cd44(-C) and -71 C>T occurred both at

Table 5.1. The β- molecular spectrum observed in the 7 studied regions in Oman. Determinants observed for the first time among Omani are highlighted.

Mutation Allele	HGVS	Musandam	Batinah	Muscat	Dhahira	Dakhiliya	Sharqiya	Dhofar	Total
IVS-I-5 G>C	HBB:c.92+5G>C	30	42	120	5	14	15		226
Cd44 (-C)	HBB:c.135delC	1	10	12	2	1	15		41
5′ (-71) C>T	HBB:c.-121C>T			34	2	5			41
Cd121 GAA>CAA	HBB:c.364G>C	5	4	13	2	3			27
IVS-I-128 T>G	HBB:c.93-3T>G	2	1	14			1		18
Cd129 TGC>TGT	HBB:c.389C>T	1		12	2				15
Cd26 GAG>AAG	HBB:c.79G>A	1	4	6		1	2		14
Cd5 (-CT)	HBB:c.17_18delCT	4	4	3		2			13
IVS-I-3′ (-25bp del)	HBB:c.93-21_96del		6	7					13
Cd58 CCT>CAT	HBB:c.176C>A			6	5		2		13
IVS-II-1 G>A	HBB:c.315+1G>A	2	6	3		1			12
Cd39 CAG>TAG	HBB:c.118C>T		8	1		1	2		12
Cd29 GGC>GGT/Cd58 CCT>CGT	HBB:c.90C>T/HBB:c.176C>G							12	12
IVS-I-1 G>A	HBB:c.92+1G>A	4	5	1					10
5′ (-101) C>T	HBB:c.-151C>T			6	1				7
5′ (-88) C>A	HBB:c.-138C>A	3		3					6
Cd15 TGG>TAG	HBB:c.47G>A			3			2		5
Cd121 GAA>AAA	HBB:c.364G>A		1	4					5
Cd30 AGG>ACG	HBB:c.92G>C	3		1					4
Cd(8/9) +G	HBB:c.27_28insG	1		2					3
IVS-II-849 A>G	HBB:c.316-2A>G			3					3
Cd6 GAG>AAG	HBB:c.19G>A			3					3
Cd8 (-AA)	HBB:c.25_26delAA	1	1	1					3
3′(+108) - 3′(+112) 5nt del	HBB:c.+108_+112delAATAA			2					2
IVS-I-6 T>C	HBB:c.92+6T>C	1		1					2
Cd10 GCC>GTC	HBB:c.32C>T			1		1			2
Cd22 GAA>TAA	HBB:c.67G>T			1					1
Cd63/37 (- T)	HBB:c.112delT				1				1
3′(+113) A>G	HBB:c.+113A>G		1						1
Cd30 AGG>AAG	HBB:c.92G>A			1					1
IVS-I-110 G>A	HBB:c.93-21G>A						1		1
Total (no. of indep. alleles)		59	93	264	20	29	40	12	517

8% while HbD Cd121 G>C was found at 5%. The other mutations were IVS-I-128 T>G and Hb La Desirade C>T, occurring at 3.5% and 2.9% respectively. HbE Cd26 G>A was found at 2.7% while Cd5(-CT), IVS-I-3'(-25bp del) and Hb-Sheffield Cd 58 C>A were observed both at 2.5%. The 21 other mutations accounted for the remaining 18.6%.

In Muscat, 27 β-alleles were found. The distributions of other β-thal alleles are shown in (Table 5.1). The origin of each mutation is described in Table 2 along with frequencies observed in the 3 countries/regions neighbouring Oman; Southern Iran (Hormozgan), United Arab Emirates and Eastern Saudi Arabia. Yemen was not included as no molecular data on beta-thalassemia were available as yet.

DISCUSSION

Although previous reports did examine the molecular spectrum of β-globin gene mutations in Oman, they were somehow limited in sample size and ethnic composition (1, 2). In addition, our samples have been randomly collected from 7 different regions, expanding by three regions from our pervious study (1). Therefore the present survey gives a better picture regarding the β-thalassemia spectrum and the distribution in a country of multi-ethnic tribes.

When comparing with neighbouring countries, the spectrum of β-thalassemia reported in our native Omani cohort (32 mutations, n=446) is higher than that reported in Eastern Saudi Arabia (14 mutations, n=196) (3) and UAE (25 mutations, n=412) (4). The most common mutation reported in the Eastern Province of Saudi Arabia was the Cd39 C>T followed by the IVS-II-1(G>A), IVS-I-5(G>C), IVS-I-25 bp deletion, and IVS-I-6 (T>C) which is also found in United Arab Emirates and Oman with varying allele frequencies (3). Among UAE nationals, the most frequent mutation found was the IVS-I-5(G>C) followed by the IVS-I-3' 25 bp del allele (4). In the Yemenis (n=10) living in Saudi Arabia, only the IVS-I-110(G>A) and IVS- II-1(G>A) mutations were identified (5). Data from Yemen are only preliminary. Although overall 52 different β-thalassemia mutations have been reported from different parts of Iran (6), the most predominant mutations found by the Iranian studies were IVS-II-I (G>A) in the north and IVS-I-5 (G>C) in the south (7, 8). The Iranian region nearest to Oman is Hormozgan. The β-thalassemia molecular spectrum of Hormozgan (9) describing 19 different mutations in 155 β-thal cases was used in our comparative study (Table 5.2).

The genetic heterogeneity of the native population shows the presence of Mediterranean, Asian Indian, Kurdish, Iranian and Turkish mutations that reflects the historical background of Oman. The Asian-Indian substitution at IVS-I-5(G>C), is the most common mutation in the UAE, Hormozgan and Oman but it is the third frequent in Eastern Saudi Arabia. Unlike our previous study where the IVS-I-5 G>C mutation was reported to constitute about 73 % of all the mutations in the Omani population studied (1), the present results revealed that IVS-I-5(G>C) constitutes about 43% of the mutations while the remaining 57% of the population carried a heterogeneous number of different mutant alleles (Table 5.1). Certain mutations occur in low frequencies and are tribe specific, indicating a founder effect. Alternatively some common mutations may vary in frequency between tribes as a consequence of bottle-necks which may wipe out certain mutations originally present in the founder population on one hand and increase the frequency of other mutations on the other hand. In the neighborhood of Muscat, immigration and trade

Table 5.2. The β-mutation frequency observed in Oman in comparison to its neighbouring countries; Hormozgan (Iran) (13), Eastern Saudi Arabia (5) and UAE (12). The most common mutation in each country is depicted in bold. 12 mutations described in Omani but not in other nationalities are highlighted in gray. β-determinants observed in other countries but not observed in Oman were not included in the table.

		Oman	Hormozgan	East KSA	UAE
Mutation Allele	**Origin**	**n=446**	**n=155 (9)**	**n=196 (3)**	**n=412 (4)**
IVS-I-5 G>C	Asian Indian	**43.7**	**69**	13.3	**44.5**
Cd44 (-C)	Kurdish	7.9	2.5	0.5	0.7
5′ (-71) C>T	Omani	7.9			
Cd121 GAA>CAA	Indian/Pakistani	5.2			2.2
IVS-I-128 T>G	Punjapi	3.5			
Cd129 TGC>TGT	Black	2.9			
Cd26 GAG>AAG	Asian Indian	2.7			0.1
Cd5 (-CT)	Mediterranean	2.5	2	3.1	2.1
IVS-I-3′ (-25bp del)	Asian Indian	2.5	1	13	8.6
Cd58 CCT>CAT	British/Omani	2.5			
IVS-II-1 G>A	East Mediterranean	2.3	9.6	22.2	2.8
Cd39 CAG>TAG	West Mediterranean	2.3	2.4	**25**	2.2
Cd29 GGC>GGT/Cd58 CCT>CGT	Omani	2.3			
IVS-I-1 G>A	Mediterranean	1.8		3.8	
5′ (-101) C>T	Turkish	1.4			0.2
5′ (-88) C>A	Kurdish	1.2	0.34		1.1
Cd15 TGG>TAG	Asian Indian	1	0.34		0.9
Cd121 GAA>AAA	Arabian/African	1			
Cd30 AGG>ACG	African	0.8	0.7		2.1
Cd(8/9) +G	Asian Indian	0.6	0.34	1.5	3
IVS-II-849 A>G	Black	0.6			
Cd6 GAG>AAG	Black	0.6			
Cd8 (-AA)	Turkish	0.6	3.4	2.1	2.2
3′(+108) - 3′(+112) 5nt del	Arabian	0.4			
IVS-I-6 T>C	West Mediterranean	0.4	0.34	7.1	1.5
Cd10 GCC>GTC	Iraqi	0.4			
Cd22 GAA>TAA	Reunion Island	0.2			
Cd36/37 (- T)	Kurdish/Iranian	0.2	0.7	0.5	0.1
3′(+113) A>G	Kurdish	0.2	2		0.4
Cd30 AGG>AAG	Bulgarian	0.2			
IVS-I-110 G>A	East Mediterranean	0.2		2.6	

may have had a considerable contribution to the variety of Asian and African beta-thal alleles as demonstrated in Table 5.2. The broad range of mutations identified in our study suggests a large effect of gene flow attributable to historical migration patterns.

Accurate characterization of the mutation at the DNA level is necessary during premarital counselling. Therefore it is essential to describe the molecular basis of the mild β^+ thalassemia alleles such as -71 C>T, IVS-I-128 T>G and Hb La Desirade Cd129 C>T since these mutations may cause problems when risk assessment is done during premarital genetic counselling. This is because these cases when accompanied with HbS, show an almost equal ratio of Hb A and Hb S (50:50) resulting in an asymptomatic phenotype as observed in previously studied cases in Omani $\beta^S/\beta^{-71C>T}$ patients (10). Moreover, variants such as Hb Sheffield Cd 58 C>A which co-elutes in the same Hb A_2 window as HbE Cd 26 G>A on high performance liquid chromatography (11) can lead to a wrong interpretation and consequently mis-diagnosis. Differentiating between mild conditions such as HbD Cd121 G>C homozygotes and compound heterozygotes (HbD/β-thalassemia) is important if the other partner is a carrier of β-thalassemia trait as there is a 25% risk of having a child affected with a severe β-thalassemia major (12). Hemoglobin variants should always be genotyped to differentiate between homozygotes and compound heterozygotes with beta-thalassemia for effective genetic counselling.

The only available option for prevention of severe hemoglobinopathies in Oman is through early diagnosis and genetic counselling. Thus molecular investigations and genotype / phenotype correlation are essential to reveal the exact β-thalassemia mutations and to make a phenotype prediction when possible. Our molecular studies show that the β-thalassemia mutations present in Oman vary in severity ranging from very mild β^+ form to severe cases, taking all studies up to date into account, at least 35 determinants are present in the country (1,2,10,11).

In conclusion, each country and region has its own prevalence and spectrum of β-thalassemia defects with a handful of common mutations and several less frequent or rare ones. Therefore, extensive knowledge on the heterogeneity of β-thalassemia mutations is needed to offer genetic counselling as a service to each population and in the highly consanguineous Omani population in particular. Finally, novel mutations will continue to be identified as genetic analysis of β-thalassemia is performed at a national level.

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