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RESEARCH ARTICLE

Molecular Bases of β-Thalassemia in the Eastern Province of Saudi Arabia

Amein K. Al-Ali,[1] Suad Al-Ateeq,[1] Burhan W. Imamwerdi,[2] Saleh Al-Sowayan,[3] Mohammed Al-Madan,[4] Fahad Al-Muhanna,[5] Laila Bashaweri,[3] and Foad Qaw[1]

[1]*Department of Biochemistry, College of Medicine, King Faisal University, PO Box 2114, Dammam 31451, Saudi Arabia*
[2]*Department of Medical Laboratory Technology, College of Medicine, King Faisal University, Dammam 31451, Saudi Arabia*
[3]*Department of Pathology, College of Medicine, King Faisal University, Dammam 31451, Saudi Arabia*
[4]*Department of Pediatrics, College of Medicine, King Faisal University, Dammam 31451, Saudi Arabia*
[5]*Department of Internal Medicine, College of Medicine, King Faisal University, Dammam 31451, Saudi Arabia*

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β-thalassemia is a group of heterogeneous recessive disorders common in many parts of the world. Al-Qatif and Al-Hassa oases in the Eastern Province of Saudi Arabia are regions known for high frequency of these disorders. Using two molecular methods, based on multiplexing-amplification refractory system and reverse hybridization principles, the spectrum of β-thalassemia in the region was studied. Sixty-nine subjects with known β-thalassemia disease and volunteers with high hemoglobin A_2(HbA_2) and low mean corpuscular volume (MCV) were included in this study. Ten mutations were detected in 91% of the subjects under study. Six of these mutations had previously been observed while the other four mutations are reported here for the first time. In addition, four of the mutations accounted for 76.8% of the subjects studied. IVSII-1 (G >A), IVSI-5 (G > A), and codon 39 (C >T) mutations were found to be the most frequent. However, the frequencies of different mutations reported here are slightly different from those reported earlier. A number of these mutations were also found in the neighboring countries, which can be explained in terms of gene flow.

INTRODUCTION

β-thalassemia is a group of heterogeneous autosomal recessive disorders due to the absence or reduced synthesis of the β-globin chain [1]. Consequently, the excess production of α-globin chain leads to its precipitation within the red blood cells causing ineffective erythropoiesis [2, 3]. Extensive work in the last two decades has led to the elucidation of the spectrum of these monogenic disorders [4]. Over 200 different mutations leading to β-thalassemia have been characterized worldwide [3]. A varied clinical expression is exhibited by homozygotes and compound heterozygotes [5]. The majority of these mutations are due to small nucleotide substitutions and deletions [3]. However, the mutations are population specific, and common and rare mutations are found in each population [6]. In the Middle East codon 39 (C > T), IVSI-110 (G > A), IVSI-1 (G > A), IVSI-6 (T > C), IVSII-1 (G > A), codon 5(-CT), and IVSI-5 (G > C) mutations account for more that 90% of β-thalassemia mutations in the region [7, 8]. However, these mutations differ in numbers and frequencies between different populations of the Middle East. Over fifty different mutations have been identified in the Arab populations reflecting the heterogeneity of these populations. In order to prevent transmission of the mutation, carriers of β-thalassemia need to be identified since their offspring are at risk of inheriting the mutation. Therefore, this work was carried out to study the spectrum of mutations of β-thalassemia in the Eastern Province of Saudi Arabia where inherited blood disorders are common.

METHODS

Over a period of two years, sixty-nine blood samples were randomly collected from unrelated Saudi male and female individuals with β-thalassemia attending King Fahad Hospital of the University, Al-Khobar, and from volunteers with HbA_2 > 3% (50 volunteers, 19 patients). After written informed consent was obtained, 3 mL of whole

Correspondence and reprint requests to Amein K. Al-Ali, Department of Biochemistry, College of Medicine, King Faisal University, PO Box 2114, Dammam 31451, Saudi Arabia, E-mail: ameinali@hotmail.com

Table 1. β-thalassemia alleles identified in the population of the Eastern Province of Saudi Arabia.

Mutation	Mutation class	Origin	Allele frequency (%)
IVSII-1 (G > A)	Splice site	Mediterranean	27.5
IVSI-5 (G > C)	Consensus mutation	Asian	23.2
Codon 39 (C > T)	Nonsense	Mediterranean	20.3
IVSI-1 (G > A)	Splice junction	Mediterranean	5.8
IVSI-6 (T > C)	Consensus splice site	Mediterranean	4.4
IVSI-25 bp	Splice junction	Asian	4.4
Codon 5(-CT)	Frameshift	Mediterranean	1.5
Codon 36/37(-T)	Frameshift	Kurdish/Iranian	1.5
Codon 44-C	Frameshift	Kurdish	1.5
IVSII-745 (C > G)	Consensus splice site	Mediterranean	1.5

blood was collected in EDTA-coated tubes. Complete blood count was determined using Coulter Micro Diff II machine and HbA_2 was determined by affinity chromatography. Genomic DNA was extracted from peripheral blood leukocytes according to standard procedures [9]. Mutation detection was carried out by the method of Fortina et al, which is based on the combination of multiplexing and amplification refractory system [10]. Five common mutations were screened by four separate reactions containing upstream primer and either four normal or four mutant primers. Polymerase chain reaction (PCR) mixtures contained 1 μg of genomic DNA, 100 mM Tris HCl (pH 8.3), 50 mM KCl, 100 μM dNTP mixture, 1.5 mM $MgCl_2$, and 0.01%(w/v) gelatin in a total volume of 50 μL. The mixtures were heated for 5 minutes at 95°C followed by the addition of four units of *Taq* DNA polymerase. Twenty-five PCR cycles of 95°C for 1 minute and combined annealing and extension at 66°C for 2 minutes with the last cycle of 3 minutes at 66°C were carried out. The β- globin strip A^{assay} kit was used to confirm the results obtained by the multiplex PCR method as well as detecting those mutations not covered by the above method [11]. In this method, PCR amplification was carried out using biotinylated primers, followed by hybridization of the PCR product to a test strip containing allele-specific oligonucleotide probes immobilized as an array of parallel lines. Bound biotinylated sequences were detected using streptavidin phosphatase and color substrate.

RESULTS AND DISCUSSION

β-thalassemias are severe congenital disorders caused by mutations in the β-globin gene resulting in the absence or reduced synthesis of the β-globin chain. This deficit leads to precipitation of excess α-globin chains resulting in the formation of inclusion bodies. In homozygotes and compound heterozygotes, this leads to a life-long dependency on blood transfusions to maintain satisfactory levels of hemoglobin along with iron chelation therapy to combat iron overload. Gene frequency for thalassemia differs markedly among different populations, reaching 10% in certain areas. Over 200 different mutations have been elucidated worldwide. However, in each population a number of common mutations (usually about 5–10) exist which account for more than 90% of all mutations [12]. β-thalassemia is endemic in the Arab countries including the countries of the Gulf region [8]. In Al-Qatif and Al-Hassa oases in the Eastern Province of Saudi Arabia, the frequency of hemoglobin S and both β- and α-thalassemia has been reported to be high [13, 14]. A number of reports have described the type of mutations that are common among the population of this area [15, 16, 17]. However, in the present study we report additional mutations which have not been reported earlier due to either limited sample size or the limitations of the procedures used. Ninety percent of all samples included in this study which had high HbA_2 (> 3%) and low MCV (< 70 ft) were shown to be homozygous or heterozygous for one of the β-thalassemia mutations detected by our procedures. Ten mutations were identified in all subjects studied (Table 1). Six of these mutations (IVSII-1 (G > A), codon 39 (C > T), IVSI-5 (G > C), IVSI-25 bp, IVSI-1 (G > A), and codon 44(-C)) have previously been identified in the Eastern Province population [15, 16, 17]. Four of the mutations (codon 5 (-CT), IVSII-745 (G > C), IVSI-6 (T > A), and codon 36/37 (-T)) are reported here for the first time in this population. Four of the mutations account for 76.8% of subjects studied. IVSII-1 (G > A) mutation was the most frequently encountered with a frequency of 27.5%. This was followed by IVSI-5 (G > C) with a frequency of 23.2%, codon 39 (C > T) with a frequency of 20.3%, and IVSI-1 (G > A) with a frequency of 5.8%. All other detected mutations had a genotype frequency of 4.4% or less.

Most of these mutations are found in neighboring countries with varying frequency. This is in line with studies in other parts of the world which have shown that gene flow due to population migration is common. Although IVSII-1 (G > A) has been previously reported to have an allele frequency of 15.1%, our study indicates that in fact it is the most common mutation (27.5%) in this area [17]. This mutation is also the most common in neighboring Kuwait at a frequency of 29% [18]. Codon 39 (C > T) which is found in western Mediterranean countries and in

neighboring Bahrain (24%) has been reported previously in the Saudi population at a high frequency [17, 18, 19]. Interestingly, the IVSI-5 (G > C) mutation has been reported at a very low frequency of 1.8% for the Eastern Province population [17] and at a higher frequency of 12% for the country as whole [15], in comparison to 23.2% in the present study. Although it is difficult to speculate on these discrepancies, it is worth mentioning that this mutation is the most frequent and widespread in the neighboring regions. The frequency of IVSI-5 (G > C) mutation in the Western Province of Saudi Arabia, Bahrain, Kuwait, UAE, and Oman are 22.5%, 16.2%, 18%, 55%, and 62%, respectively [8, 19, 20, 21]. Therefore, it is not surprising that the IVSI-5 (G > C) mutation is found at high frequency in the Eastern Province due to gene flow. Both IVSI-1 (G > A) and IVSI-6 (T > A), which are Mediterranean in origin, are also found at lower frequencies in the population of the Eastern Province. Although IVSI-25 bp mutation is found at high frequency in neighboring Bahrain (36%), in the population of the Eastern Province it is only found at low frequency (2.7%). Codon 44(-C) mutation, which is of Kurdish origin, is found in the Gulf country of Oman and at a low frequency in the present population of the Eastern Province. Codon 36/37(-T) which is of Kurdish/Iranian origin and IVSII-745 (G > C) and IVSI-6 (T > A) which are of Mediterranean origin are also found in the present population but at a lower frequency. Therefore, it is not surprising that such mutations are found in the Eastern Province due to the close proximity of these populations.

In summary, the present report identifies four new mutations in the population of the Eastern Province. The frequencies of the mutations reported in the present study are in line with the frequencies of these mutations reported in the surrounding populations.

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