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ORIGINAL ARTICLE

MOLECULAR BASIS OF β-THALASSEMIA IN THE WESTERN PROVINCE OF SAUDI ARABIA: IDENTIFICATION OF RARE β-THALASSEMIA MUTATIONS

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□ *This study aimed at the identification of the spectrum of mutations in patients with β-thalassemia (β-thal) in the western province of Saudi Arabia. Screening for the mutations was done using the polymerase chain reaction-amplification refractory mutation system (PCR-ARMS) technique to test for 12 mutations, and direct automated DNA sequencing for the unknown samples. The study included 172 patients; of these 15 patients had sickle cell anemia and one Hb S [β6(A3)Glu→Val, GAG>GTG]/β-thal. A total of 23 mutations were identified to cause the disease in the western area. Seven common mutations were responsible for the β-thal alleles in 78% of patients and could be detected by the ARMS technique: IVS-II-1 (G>A), IVS-I-110 (G>A), IVS-I-5 (G>C), codon 39 (C>T), codon 26 (G>A) [Hb E or β26(B8)Glu→Lys, GAG>AAG], frameshift codons (FSC) 8/9 (+G), and IVS-I-1 (G>A). DNA sequencing of uncharacterized alleles detected eight less common mutations: FSC 41/42 (–TCTT), IVS-I 25 bp deletion, codon 37 (G>A), FSC 44 (–C), Cap site+1 (A>C), IVS-I-6 (T>C), FSC 5 (–CT) and IVS-I-1 (G>T), and eight rare mutations: −87 (C>G), initiation codon −1 (T>G), codon 15 (G>A), FSC 16 (–C), FSC 20/21 (+G), codon 27 (G>A), IVS-I-130 (G>C) and IVS-II-837 (A>C). Four alleles were normal by DNA sequencing. Genetic heterogeneity was observed in this study, 10 mutations were of Asian or Asian/Indian origin, two were Kurdish, one Chinese, one Turkish, one Saudi, and the remainder were of Mediterranean origin. The presence of a large population of immigrants in the*

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western province is responsible for the great heterogeneity at the molecular level, and for the difference observed in the frequencies of mutations from those reported in the eastern province of Saudi Arabia. Screening for β-thal mutations using PCR-ARMS for the seven most frequent mutations in the Saudi population followed by DNA sequencing of the unknown alleles could be useful for the implementation of a strategy for carrier detection and preimplantation genetic diagnosis in high risk families.

Keywords β-Thalassemia (β-thal), Spectrum of β-thalassemia mutations, Polymerase chain reaction-amplification refractory mutation system (PCR-ARMS), Direct DNA sequencing

INTRODUCTION

β-Thalassemia (β-thal) and sickle cell anemia are considered a major public health problem in the Mediterranean, African and Asian regions. Researchers in Saudi Arabia have revealed the wide distribution of the Hb S [β6(A3)Glu→Val, G*A*G>G*T*G] and β-thal genes in Saudi Arabia. The Hb S, α- and β-thal gene frequencies have been estimated as 0.005-0.145, 0.01-0.40, and 0.01-0.15, respectively in various areas of Saudi Arabia (1). A high rate of occurrence of these genes has been reported in the eastern and western provinces, particularly in the southwestern province (2). The estimated prevalence of thalassemia trait in El-Hassa during a premarital screening program revealed a carrier rate of 3.4% with a high Hb A_2 level and microcytic hypochromic anemia (3). Another study in Al-Qassim has shown a prevalence rate of β-thal and sickle cell traits of 0.165 and 0.252%, respectively (4).

Defects in the β-globin gene lead to a decreased rate (β^+) or absence (β^0) of production of the β-globin chain of hemoglobin (Hb), and excess precipitation of α-globin chains in red blood cells causing different degrees of hemolytic anemia (5). To date, more than 200 mutations causing β-thal have been reported in the Human Gene Mutation Database (www.hgmd.cf.ac.uk). However, in each population, a handful of ethnic group-specific alleles accounts for roughly 90-93% of the β-thal alleles. Considerable heterogeneity has been reported at the molecular level of β-thal in different studies in Mediterranean populations (6–8). A few studies have been reported to explore the genetic basis of β-thal in Saudi Arabia (9–12). Therefore, we opted to explore the spectrum of mutations underlying this disease in a group of β-thal major (β-TM) patients in the western province of Saudi Arabia, utilizing both the polymerase chain reaction-amplification refractory mutation system (PCR-ARMS) technique and direct automated DNA sequencing for the uncharacterized alleles.

The study aimed at identifying the spectrum of mutations causing β-thal in the western province of Saudi Arabia. We also wanted to investigate the usefulness of the PCR-ARMS technique followed by DNA sequencing as diagnostic tools that could be applied for carrier detection and prenatal diagnosis.

SUBJECTS AND METHODS

The study included 172 samples randomly collected from unrelated Saudi patients with β-thal major (β-TM) and sickle cell anemia. Patients were referred to the Centre of Excellence in Genomic Medicine Research (CEGMR), Jeddah, Saudi Arabia for molecular diagnosis from the Paediatric Haematology Clinic, King AbdulAziz University Hospital, Jeddah, Saudi Arabia and other hospitals in the western region. After a written informed consent was obtained, 3 mL of whole blood was collected in EDTA as anticoagulant. Patients were diagnosed by both clinical evaluation and hematological examination. Two families who had a previous history of an affected child presented for prenatal diagnosis early in pregnancy, and another family for carrier diagnosis of an affected sibling.

Genomic DNA Extraction

Genomic DNA was extracted from the leukocytes of each subject using the salting out technique (13) or the DNA blood mini kit (Qiagen, Hilden, Germany) following the manufacturer's instructions.

Mutation Detection

Screening for point mutations in the β-globin gene was done using the allele-specific PCR-ARMS as previously described (14,15). Sequence specific PCR primers were used where the 3′ end of one primer is designed to match the normal sequence (N) and the other primer designed to match the mutant sequence (M) for each tested mutation.

Polymerase chain reaction amplification for the diagnosis of 12 of the most common β-globin gene mutations in the Mediterranean and Asian region included: IVS-I-110 (G>A), IVS-II-1 (G>A), IVS-I-5 (G>C), codon 39 (C>T), frameshift codon (FSC) 5 (–CT), IVS-I-1 (G>A), IVS-I-6 (T>C), FSC 41/42 (–TCTT), IVS-II-745 (C>G), IVS-II-654 (C>T), Cap site +1 (A>C) and the Hb S mutation. Multiplex PCR was done in three or four reactions using primers specific for the mutant alleles in the following combination: IVS-I-110, IVS-II-1, IVS-I-1 (reaction 1); codon 39, IVS-I-5, FSC 5 (reaction 2); codon 26 [Hb E or β26(B8)Glu→Lys, *G*AG>*A*AG], FSC 41/42 (reaction 3); IVS-II-654, IVS-II-745 (reaction 4). Primers specific for the IVS-I-6 and Hb S β6 GAG>GTG (A>T) mutations are included in reaction 1 whenever required. Amplified products were run on 2% agarose gel and stained with ethidium bromide for visualization.

Automated DNA sequencing analyses were done to confirm results of the multiplex PCR-ARMS technique, and to diagnose the molecular defect in cases proven not to have any of the studied mutations. Amplification of genomic DNA was performed using two sets of primers: one pair of primers

amplified the β-globin gene starting from the promoter region at −160 nucleotides (nt) 5′ upstream from the first exon to IVS-II, 115 nt downstream from the beginning of the second intron. The other primer pair started from IVS-II, 600 to 197 nt 3′ to the gene. The resulting fragments, 770 and 560 bp, contained most of the sites specific for the Mediterranean and Southeast Asian populations. The PCR products were directly sequenced on the ABI PRISM™ 3130 DNA sequencer (Applied Biosystems, Foster City, CA, USA) using the BigDye terminator kit (Applied Biosystems) according to the manufacturer's instructions.

RESULTS

The study included 172 patients with β-thal ascertained to be from families with at least one affected child with β-TM or sickle cell anemia. There were 156 β-thal patients (148 probands and eight affected siblings) and 15 patients with sickle cell anemia and one Hb S/β-thal. There were 105 males and 67 females, a ratio of 1.6:1, and their ages ranged from 1 to 40 years old.

Samples showing negative results were subjected to DNA sequencing using two sets of primers. The first amplified product spans most hot-spots for mutations in the first exon, first intron and the second exon as well as splicing regions. The second primer pair amplifies the 3′ end of the second intron, third exon and the polyadenylation site (poly A) tail. This design covers 95% of mutations in the β-globin gene.

The results of the mutation screening are shown in Table 1; a total of 23 β-thal mutations could be detected by this technique; however four alleles (1.4%) were normal by the ARMS technique and had normal sequences by DNA sequencing. A high rate of homozygous mutations (93/142) (65%) was observed in this study (Table 2) indicating a high rate of consanguineous marriages.

Seven mutations accounted for 78% of the total number of mutations identified in this population and they are known to be common in Mediterranean as well as Asian regions: IVS-I-5, IVS-II-1, codon 39, IVS-I-110, IVS-I-1, FSC 8/9 and codon 26 (Hb E). The last two mutations (FSC 8/9 and codon 26) were not tested in the multiplex reactions and were diagnosed by DNA sequencing in the first phase of the study, and were included in the multiplex reaction of the PCR-ARMS technique thereafter. There were eight less common mutations: IVS-I 25 bp deletion, FSC 41/42, nonsense codon 37 (G>A), Cap site +1, FSC, FSC 5 (–CT), FSC 44 (–C), IVS-I-6, and IVS-I-1 accounted for 14% of the total mutations. Sickle cell mutations were detected in 31 alleles, and were not included in the allele frequency. Samples with Hb E and Hb S mutations were received without any information about the results of Hb electrophoresis and were only detected by DNA sequencing. Two mutations, IVS-II-745 and IVS-II-654, were not found in

TABLE 1 β-Thalassemia Alleles identified in the Western Province Compared to the Eastern Province of Saudi Arabia

Mutation	β Type	Origin	Present Study $n = 297$ (%)	Saudi Arabia (9)[a] $n = 186$ (%)	Eastern Saudi Arabia (12)[a] $n = 138$ (%)	Western Saudi Arabia (11)[a] $n = 62$ (%)
IVS-II-1, G>A	β^0	Mediterranean	52(17.50)	(12.90)	(27.50)	(15.10)
IVS-I-110, G>A	β^+	Mediterranean	40(13.50)	(26.90)	–	–
IVS-I-5, G>C	β^+	Asian Indian	57(19.20)	(12.90)	(23.20)	(1.90)
Codon 39, C>T	β^0	Mediterranean	23(7.70)	(12.90)	(20.30)	(32.10)
IVS-I-5, G>T	β^+	Mediterranean	–	–	–	(1.90)
Codon 26, G>A (Hb E)	β^E	Southeast Asian	27(9.10)	–	–	–
IVS-I-1, G>A	β^0	Mediterranean	12(4.00)	–	(5.80)	–
FSC 8/9, +G	β^0	Asian Indian	22(7.40)	(1.07)	–	(3.80)
IVS-I, 25 bp deletion	β^0	Asian Indian	8(2.70)	(12.90)	(4.40)	(22.60)
Codon 37, G>A	β^0	Saudi Arabian	7(2.40)	–	–	–
FSC 41/42, –TCTT	β^0	Asian Indian	7(2.40)	–	–	–
Cap site +1, A>C	β^+	Asian Indian	4(1.40)	(1.07)	–	–
FSC 5, –CT	β^0	Mediterranean	3(1.00)	–	(1.50)	–
FSC 6, –A	β^0	Mediterranean	–	(4.30)	–	–
IVS-I-6, T>C	β^+	Mediterranean	3(1.00)	–	(4.40)	–
FSC 8, –AA	β^0	Turkish	–	–	–	(15.10)
FSC 44, –C	β^0	Kurdish	6(2.00)	–	(1.50)	(7.50)
IVS-I-1, G>T	β^0	Asian Indian; Chinese	3(1.00)	–	–	–
FSC 36/37, –T	β^0	Kurdish; Iranian	–	–	(1.50)	–
IVS-II-745, C>G	β^+	Mediterranean	–	–	(1.50)	–
–87, C>G	β^+	Mediterranean	1(0.34)	–	–	–
Codon 27, G>T (Hb Knossos)	β^+	Mediterranean	1(0.34)	–	–	–
Codon 15, G>A	β^0	Asian Indian	1(0.34)	–	–	–
Codon −1, T>G	β^0	Chinese	1(0.34)	–	–	–
FSC 20/21, +G	β^0	Israili	1(0.34)	–	–	–
Codon 16, –C	β^0	Asian Indian	2(0.67)	–	–	–
IVS-I-130, G>C	β^0	Turkish	2(0.67)	–	–	–
IVS-II-837, A>C	β^+	Asian Indian	2(0.67)	–	–	–
Normal sequence			4(0.67)	–	–	–
Uncharacterized			8(2.70)	–	–	–

[a] References.

TABLE 2 Genotypes of β-Thalassemia Patients in the Western Province of Saudi Arabia

β-Thalassemia Mutations	β Type	*n*	%
Homozygotes			
IVS-II-1 (G>A)	β^0	22	14.86
IVS-I-5 (G>C)	β^+	22	14.86
IVS-I-110 (G>A)	β^+	16	11.00
Codon 39 (C>T)	β^0	9	6.10
FSC 8/9 (+G)	β^0	9	6.10
IVS-I, 25 bp deletion	β^0	3	2.00
FSC 44 (–C)	β^0	3	2.00
Codon 37 (G>A)	β^0	2	1.40
FSC 41/42 (–TCTT)	β^0	2	1.40
IVS-I-1 (G>T)	β^0	1	0.70
IVS-I-1 (G>A)	β^0	1	0.70
IVS-I-6 (T>C)	β^+	1	0.70
IVS-I-130 (G>C)	β^+	1	0.70
IVS-II-837 (A>C)	β^+	1	0.70
Double Heterozygotes			
Codon 26(G>A)/IVS-I-5(G>C)	β^E/β^0	11	7.40
Codon 26(G>A)/IVS-I-1(G>A)	β^E/β^0	5	3.40
Codon 26(G>A)/IVS-II-1(G>A)	β^E/β^0	5	3.40
IVS-I-110(G>A)/IVS-I-1(G>A)	β^+/β^0	3	2.00
Cap site +1(A>C)/FSC 8/9(+G)	β^+/β^0	2	1.40
IVS-I-110(G>A)/FSC 5(–CT)	β^+/β^0	2	1.40
IVS-I-110(G>A)/codon 37(G>A)	β^+/β^0	2	1.40
FSC 16(–C)/codon 26(G>A) (Hb E)	β^0/β^E	2	1.40
Codon 39(C>T)/IVS-I-1(G>A)	β^0/β^0	1	0.70
Codon 39(C>T)/FSC 8/9(+G)	β^0/β^0	1	0.70
Codon 39(C>T)/IVS-I (25 bp deletion)	β^0/β^0	1	0.70
Codon 39(C>T)/FSC 41/42(–TCTT)	β^0/β^0	1	0.70
Cap site +1(A>C)/FSC 41/42(–TCTT)	β^+/β^0	1	0.70
Cap site +1(A>C)/codon 15(G>A)	β^+/β^0	1	0.70
IVS-I-110(G>A)/IVS-I-6(T>C)	β^+/β^+	1	0.70
IVS-I-5(G>C)/FSC 5(–CT)	β^+/β^0	1	0.70
IVS-I-1(G>A)/codon 37(G>A)	β^0/β^0	1	0.70
IVS-I-1(G>A)/FSC 41/42(–TCTT)	β^0/β^0	1	0.70
IVS-II-1(G>A)/–87(C>G)	β^0/β^+	1	0.70
Codon 26(G>A)/codon 39(C>T)	β^E/β^0	1	0.70
Codon 26(G>A)/FSC 8/9(+G)	β^E/β^0	1	0.70
Codon 39(C>T)/codon 27(G>T) (Hb Knossos)	β^0/β^+	1	0.70
Codon −1(T>G)/IVS-I-5(G>C)	β^0/β^+	1	0.70
IVS-I(25 bp deletion)/codon 39(C>T)	β^0/β^0	1	0.70
FSC 20/21(+G)/?	$\beta^0/?$	1	0.70

our patients using both the PCR-ARMS and automated DNA sequencing techniques.

Rare mutations could be identified that have not been previously reported in Saudi Arabia. They are: initiation codon −1 (T>G), codon 15 (G>A), FSC 16 (–C), codons 20/21(+G), IVS-I-130 (G>C) and IVS-II-837

TABLE 3 Frequency of β-Thalassemia Alleles in the Western Province Compared With Other Populations

Mutation	β Type	Present Study %	Egyptians (6) %	Turkish (40) %	Kuwaitis (18) %	Pakistanis (23) %	Indians (25) %
IVS-I-5, G>C	β^+	19.20	–	1.10	18.80	37.70	54.50
IVS-II-1, G>A	β^0	17.50	3.00	4.70	29.00	0.70	1.60
IVS-I-110, G>A	β^+	13.50	41.00	39.30	–	–	–
Codon 26, G>A (Hb E)	β^E	8.40	–	–	–	0.50	0.14
Codon 39, C>T	β^0	7.40	1.40	3.80	7.30	0.20	–
FSC 8/9, +G	β^0	7.40	–	1.30	1.30	21.10	4.90
IVS-I-1, G>A	β^0	4.00	13.00	5.00	7.30	1.70	0.60
IVS-I, 25 bp deletion	β^0	2.70	–	–	7.30	–	0.14
Codon 37, G>A	β^0	2.40	1.40	–	–	–	–
FSC 41/42, –TCTT	β^0	2.40	–	–	–	2.70	4.80
FSC 44, –C	β^0	2.00	–	1.30	1.00	–	–
Cap site +1, A>C	β^+	1.40	–	–	–	1.20	1.60
FSC 5, –CT	β^0	1.00	3.00	2.20	–	3.10	1.80
IVS-I-1, G>T	β^0	1.00	–	–	–	9.50	6.20
IVS-I-6, T>C	β^+	1.00	13.00	10.10	7.30	–	–
Codon 16, –C	β^0	0.67	–	–	–	2.10	0.70
IVS-I-130, G>C	β^+	0.67	–	0.10	–	–	0.26
IVS-II-837, A>C	β^+	0.67	–	–	–	–	0.60
–87, C>G	β^+	0.34	–	0.80	–	–	–
Codon 27, G>T (Hb Knossos)	β^+	0.34	1.40	0.10	–	–	–
Codon 15, G>A	β^0	0.34	–	0.10	–	3.10	8.20
Codon −1, T>G	β^0	0.34	–	–	–	–	–
FSC 20/21, +G	β^0	0.34	–	–	–	–	–
FSC 6, –A	β^0	–	–	0.40	–	–	–
FSC 8, –AA	β^0	–	3.00	5.50	3.00	–	–
619 bp deletion	β^0	–	–	–	–	12.40	9.50
Codon 30, G>C	β^0	–	–	–	–	2.10	2.90
FSC 106/107, +G	β^0	–	3.00	–	–	–	–
FSC 36/37, –T	β^0	–	–	0.10	–	–	–
IVS-II-745, A>C	β^+	–	3.00	5.00	–	–	–
IVS-II-848, C>A	β^+	–	11.00	0.40	–	–	–

References are in parentheses.

(A>C). Table 3 presents the β-thal allele frequency in the Saudi population as compared to other populations.

Two families with a previously affected child presented for prenatal diagnosis. Family #134 had a child homozygous for the FSC 8/9 mutation, and the second family (#572) had an affected child doubly heterozygous for FSC 41/42 and the IVS-I-1 mutations. Genetic testing was done using DNA extracted from chorionic villus or amniotic fluid samples followed by direct automated DNA sequencing. The two fetuses were not carriers of the mutations and had a normal β-globin gene sequence. The two families were

provided genetic counseling. This is the first time that we have provided prenatal diagnosis for thalassemia at the CEGMR, King AbdulAziz University, Jeddah, Saudi Arabia.

DISCUSSION

β-Thalassemia is endemic in the Mediterranean and Middle Eastern populations including the Gulf region. The frequency of Hb S and thalassemia has been reported to be high in the eastern as well as the western regions of Saudi Arabia (16,17), although few studies have been reported for the elucidation of the molecular basis of β-thal. The main reports were from the eastern province (11,12) and the central region (Riyadh) (9,10). To the best of our knowledge, no previous studies have so far been reported in the western province for the identification of mutations in β-thal patients.

In this study, we report the spectrum of mutations in the β-globin gene in a cohort of β-TM patients in the western region of Saudi Arabia and our first experience in prenatal diagnosis. A high rate of homozygosity for the same β-thal alleles (69%) was observed in this study, as expected from the high rate of consanguineous marriages in the Saudi population.

It has been noticed that frequencies of β-globin gene mutations are different in various geographical regions all over the world and between ethnic groups, where a handful of mutations are responsible for the disease in each population. Saudi Arabia has a unique geographic position lying between the Mediterranean and Southeast Asian regions where people from several areas migrated to different regions of Saudi Arabia.

A total of 23 different mutations was identified in this study; of these 10 have been reported in the eastern region of Saudi Arabia (11,12) and are known to be common in the Mediterranean as well as the Southeast Asian regions. Genetic heterogeneity was observed more in the western region compared to the eastern province of Saudi Arabia where eight (11) and 10 (12) mutations were reported in two different studies. The larger number of mutations is thought to be due to the larger migrations in the western area.

In our study, seven mutations accounted for 78% of the studied alleles, compared to four mutations accounting for 76.8% of alleles studied in the eastern area (11,12). Four of these mutations are known to be of Mediterranean in origin: IVS-II-1, IVS-I-110, codon 39 and IVS-I-1, and the other three are Asian-Indian in origin: IVS-I-5, FSC 8/9 and Hb E (codon 26). Four mutations were observed to be most common in our study as well as other studies in Saudi Arabia, mainly IVS-II-1, IVS-I-5, codon 39 and IVS-I-110 (1,11,12,17). The most frequent mutations in the eastern region were IVS-II-1 (27.5%) followed by IVS-I-5 (23.2%). However, the IVS-I-5 mutation was the most common one in our study (19.2%) followed by IVS-II-1

(17.5%). The latter mutation was also found at high frequency in neighboring countries such as Kuwait (29%) (18). Similarly, the frequency of the IVS-I-5 is also quite common in Kuwait (18.8%) (18), Bahrain (16.7%), United Arab Emirates (55%) and Oman (61%) (8). These mutations might also be found in the eastern province due to gene flow-from neighboring countries. Mutations which are Mediterranean in origin such as codon 39 are found in Western Mediterranean countries and in Bahrain (24%) (8) and have also been reported in the Saudi population at variable frequencies in the eastern region (20.3%) (11) and (12.9%) (12) compared to 7.7% our study in the western region.

There are eight less common mutations that account for 14% of the mutations: IVS-I 25 bp deletion, FSC 41/42, nonsense codon 37, Cap site +1, FSC 5, FSC 44, IVS-I-6 and IVS-I-1. The nonsense codon 37 mutation has been previously reported in isolated cases in Saudi Arabia (19) and an Egyptian patient (6). This mutation was present in seven alleles (2.4%) in our study but was absent in the eastern province.

It was noticed in our study that Hb E (codon 26) was identified at a moderate frequency (9%) and was not reported in the eastern region; similarly the FSC 41/42, IVS-I-1 and the other seven rare mutations observed in our study were not reported in the eastern province of Saudi Arabia. Contrarily, four mutations that were reported in the eastern region [codon 6 (–A), codon 8 (–AA), codons 36/37 (–T) and IVS-II-745] were not observed in our study; they are all of Mediterranean origin except the codons 36/37 which is of Kurdish-Iranian origin.

The wide spectrum of the found mutations gave a snapshot of the genetic heterogeneity of the urban population of the western province of Saudi Arabia. The main reason is the centuries of pilgrimage (Hajj) migration as well as economic migration and settlement in the port city of Jeddah and surrounding areas from around the world. The main migrations came from India and Southeast Asia. Mutations that are known in Southeast Asia were frequently found in this study. The IVS-I-5, FSC 8/9, FSC 41/42 and IVS-I-1 mutations are observed at high frequencies in Pakistan (37.5, 25.9, 6.7, 5.4%) (20,21), North India (32.7, 22.3, 5.8, 13.8%) (22), as well as other Southeast Asian countries (23).

Two Mediterranean mutations (–87) and Hb Knossos [β27(B9) Ala→Ser, *G*CC>*T*CC; codon 27 (G>C)] are reported in our study at low frequency (0.33%) for the first time; each found on one allele in two different patients. Three patients had the Cap site +1 mutation, and are double heterozygotes for a severe β-thal mutation (FSC 8/9, FSC 41/42 and nonsense codon 15, respectively). The Cap site +1 nt is the start site for transcription and is the site of the capping modification of precursor RNA. The A>C mutation is a nearly silent carrier allele and if in a homozygous state produces values of a mild β-thal carrier. The combination of this mutation

with a severe allele can produce β-TM (24) which is the reason of the severe phenotype observed in these patients.

In this study, we present the first report of six rare mutations that have never before been reported in the Saudi population. They are known as rare mutations in Chinese and Southeast Asian populations: initiation codon −1, nonsense codon 15, FSC 16, FSC 20/21, IVS-I-130 and IVS-II-837.

The initiation codon mutation (A*T*G>A*G*G) was independently described in unrelated families from China (25), Japan (26), Korea (27) and Thailand (28). Mutations in the initiation codon are expected to change the initiation site to codons 21-22 and result in premature termination at codons 60-61 causing β-TM (28). This mutation has never been described before in the Saudi population and is present in a compound heterozygote with the IVS-I-5 mutation in one individual, and may reflect the presence of immigrants from the Asian populations.

In our study, the rare mutation FSC 16 was found to co-exist with codon 10 (C>A) in two different patients. The patients were double heterozygotes for the FSC 16/codon 26 (Hb E) mutations, suggesting that the codon 10 mutation is a rare polymorphism. The mutation at codon 10 (GC*C*>GC*A*) did not change the alanine amino acid residue, and was found to coexist with the FSC 16 mutation (29,30). However, it was suggested that the C>A substitution at codon 10 creates a sequence homologous to the normal splice site at the exon1/intron1 boundary and causes alternative splicing at the site giving a β^+ phenotype (31–33). The mutation was reported in a multicenter study in Pakistan, Sri Lanka and India; the authors suggested the codon 10 mutation is just a rare polymorphism on an ancestral allele, on which the codon 16 mutation had arisen (30).

We report one more new mutation that has never been reported in Saudi Arabia, the FSC 20/21 mutation; this mutation is rare and was first reported in Ashkenazi-Jews in Israel (34,35). The mutation has been reported in another Ashkenazi-Jewish family in Montreal (36). The members of the two families shared the same mutation and haplotype, suggesting a common ancestral origin. Haplotype analysis was not done in our family.

Another Saudi patient was homozygous for the IVS-I-130 mutation in the first intron/exon2 boundary at the acceptor splice site. This mutation is very rarely observed all over the world and was first reported in Turkey (37,38). It has been shown that the IVS-I-130 mutation changes the consensus acceptor sequence (A*G*>A*C*) at the intron1/exon 2 boundary and may prevent splicing (39) resulting in a severe β-TM phenotype.

The rare IVS-II-837 mutation was found in the homozygous state in one patient; this mutation was first reported in Asian immigrants in the UK (33) and then in South India (40). The mutation creates an alternative 3′ splice site that might be preferentially utilized resulting in abnormal splicing of

the mRNA and formation of a nonfunctional β-globin gene product. The patient in our study has a β-TM phenotype.

The present study provides data on the frequency of β-thal mutations in the western region of Saudi Arabia. To the best of our knowledge, this is the first extensive molecular study in the western province, genetic heterogeneity was observed and a larger number of rare mutations was first reported in this study. In the CEGMR, we started to offer carrier detection, premarital testing, prenatal diagnosis and genetic counseling at the CEGMR, King AbdulAziz University, Jeddah, Saudi Arabia. Results of this study can offer the basis for a program for screening carriers, premarital and pre implantation genetic diagnosis for reducing the burden of β-thal in the Saudi population.

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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