Mutations Associated with Beta-Thalassemia intermedia in Kuwait

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Key Words

 β -Thalassemia intermedia • Kuwait

Abstract

Objective: To identify the β -globin gene mutations associated with β -thalassemia (β -thal) intermedia in Kuwait. **Subjects and Methods:** Eighteen patients from 13 unrelated families, mean age 12.7 ± 8.1 years, range 4–31 years, were involved in the study. They did not require regular blood transfusion. Complete blood count and cation exchange high-performance liquid chromatography hemoglobin quantitation were carried out using standard techniques. β -Thal mutations were identified with a combination of PCR amplification, allele-specific oligonucleotide hybridization or direct DNA sequencing. The patients were also screened for the α_2 -globin gene (–3.7 kb) deletion. **Results:** Of the 13 families, 4 were homozygous for the IVS-I-II (G→A) and 4 for the IVS-I-6 (T→C) mutations, while 1 each was a compound heterozygote for the following mutation combinations: CD 8 (–AA) and –101 (C→T); IVS-I-6 (T→C) and CD 19 (A→G); IVS-II-1 (G→A) and –28 (A→C); IVS-I-110 (G→A) and $\delta\beta^0$ deletion. Therefore, homozygosity for two typically mild mutations (IVS-II-1 and IVS-I-6) accounted for 61% of the genotypes in our patients. **Conclusion:** Our results indicate that screening should commence with these two common alleles in Kuwaiti patients presenting with β -thal syndrome. Early identification of intermedia patients will avoid the complications following an unnecessary hypertransfusion program.

Introduction

β -Thalassemia (β -thal) is a heterogeneous group of disorders associated with defects within the β -globin gene locus, leading to absent (β^0) or reduced (β^+) synthesis of the β -globin chain. Inheritance of only one of these β -thal alleles is usually asymptomatic (β -thal trait), but two alleles (homozygote or compound heterozygote) would produce thalassemia major (TM) or thalassemia intermedia (TI). While TM is associated with early onset of severe anemia, which rapidly becomes transfusion dependent, TI is milder and variable. The TI patients do not require regular transfusion but may have hepatosplenomegaly and skeletal abnormalities [1–3].

TI is also quite heterogeneous at the genotype level and several molecular mechanisms have been proposed. Typically, mutations in the promoter, splice site activation, consensus sequence and polyadenylation site of the β -globin gene are associated with mild or silent thalassemia. The –158 C→T substitution in the γ -globin gene promoter, linked to frameshift codon 6 (–A), codon 8 (–AA) and IVS-II-1 (G→A) is associated with increased hemoglobin (Hb) F synthesis and patients with these mutations commonly have TI. Coinheritance of α -thal trait is also associated with TI because it reduces globin chain imbalance [4–8].

There is usually a segregation of a small number of particular β -thal mutations in discrete populations or ethnic groups [9, 10]. However, in a situation where there is significant genetic admixture or interactions, the number of mutations may be quite high. It is therefore desirable

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Table 1. Molecular and hematological characteristics of β -thal intermedia patients in Kuwait

Serial No.	Nationality	Family No.	Sex/age	Mutations	α -Globin genotype	Hb g/dl	MCV fl	MCH pg	A ₂ %	Hb F %
1	Kuwaiti	1	M/17	IVS-II-1/IVS-II-1	$-\alpha/\alpha\alpha$	9.9	85.3	23.8	1.3	98.7
2	Kuwaiti	2	M/16	IVS-II-1/IVS-II-1	$-\alpha/\alpha\alpha$	9.2	72.6	20.9	3.9	96.1
3	Kuwaiti	2	M/13	IVS-II-1/IVS-II-1	$-\alpha/\alpha\alpha$	11.0	88.8	25.8	2.7	95.9
4	Kuwaiti	3	F/31	IVS-II-1/IVS-II-1	$\alpha\alpha/\alpha\alpha$	9.0	74.5	21.2	2.0	98.0
5	Kuwaiti	4	F/4	IVS-II-1/IVS-II-1	$\alpha\alpha/\alpha\alpha$	10.0	78.3	25.4	1.0	99.0
6	Kuwaiti	5	F/13	IVS-I-6/IVS-I-6	$\alpha\alpha/\alpha\alpha$	9.0	69.7	18.3	5.7	23.5
7	Jordanian	6	F/4	IVS-I-6/IVS-I-6	$\alpha\alpha/\alpha\alpha$	9.1	60.7	16.7	3.7	31.0
8	Jordanian	7	F/18	IVS-I-6/IVS-I-6	$\alpha\alpha/\alpha\alpha$	8.7	64.5	19.0	5.2	24.1
9	Jordanian	8	M/15	IVS-I-6/IVS-I-6	$\alpha\alpha/\alpha\alpha$	8.2	63.2	17.2	6.4	12.3
10	Kuwaiti	9	M/10	-101/CD 8	$\alpha\alpha/\alpha\alpha$	10.9	59.1	17.4	5.7	25.6
11	Kuwaiti	9	M/20	-101/CD 8	$\alpha\alpha/\alpha\alpha$	9.9	60.3	17.9	5.3	22.1
12	Kuwaiti	9	M/29	-101/CD 8	$\alpha\alpha/\alpha\alpha$	11.3	58.7	17.6	7.4	23.7
13	Kuwaiti	10	F/8	CD 8/IVS-II-1	$\alpha\alpha/\alpha\alpha$	9.0	82.1	21.9	2.3	97.3
14	Kuwaiti	10	M/7	CD 8/IVS-II-1	$-\alpha/\alpha\alpha$	10.4	77.4	22.1	1.5	98.5
15	Kuwaiti	11	M/6	IVS-I-6/CD 19	$\alpha\alpha/\alpha\alpha$	7.8	54.9	18.4	5.0	24.5
16	Kuwaiti	11	M/5	IVS-I-6/CD 19	$\alpha\alpha\alpha/\alpha\alpha$	7.8	59.2	19.8	5.6	19.6
17	Iraqi	12	M/7	IVS-II-1/-28	$-\alpha/\alpha\alpha$	10.2	71.6	22.0	2.7	54.0
18	Jordanian	13	F/5	IVS-I-110/ $\delta\beta$ thal	$\alpha\alpha/\alpha\alpha$	8.2	67.3	24.5	1.1	92.7

MCV = Mean corpuscular volume; MCH = mean hemoglobin concentration; A₂ = hemoglobin A₂.

to establish a database of such mutations in every society in which β -thal is found. This not only facilitates prenatal and early childhood diagnosis, but also provides a solid basis for genetic counseling and the institution of appropriate management. Therefore, the aim of this study was to identify the β -globin gene mutations associated with β -thal intermedia in Kuwait.

Subjects and Methods

Patients referred to the Pediatric Hematology Clinic in the Mubarak and Amiri Hospitals, Kuwait, with the presumptive diagnosis of TI were eligible for inclusion into the study. The diagnosis of TI was based on the presence of chronic hypochromic, microcytic anemia with normal iron status. There were 18 patients (11 male and 7 female) from 13 families, 15 of whom were referred for investigation and the remaining 3 were siblings who were identified through family screening. Mean age was 12.1 ± 8.1 years, range 4–31 years. Other inclusion criteria included a peripheral blood picture and Hb electrophoresis pattern consistent with β -thal syndrome and a baseline Hb level of ≥ 7.0 g/dl without the need for regular blood transfusion. The immediate family was screened with complete blood count and Hb electrophoresis and both parents had to have evidence of β -thal trait for the index case to be admitted into the study.

The 18 patients were fully characterized. Blood was obtained by venepuncture from each patient, complete blood count was done with an electronic cell counter (Coulter S) and Hb quantitation was done with cation exchange high-performance liquid chromatogra-

phy [11]. DNA was isolated using the method of Poncz et al. [12]. The region of the β -globin gene was amplified with primers as previously described [13, 14]. They were screened for common β -thal mutations using allele-specific oligonucleotide hybridization. Those that were not readily identified had sequencing of the β -globin gene done using the dideoxy method of Sanger et al. [15], while one was solved by automated sequencing in Augusta, Ga., USA (F.K.) and one by gene mapping in London University Hospital, London, UK. All the patients were also screened for α -thal-2 –3.7-kb deletion using a PCR method [16]. Although full haplotyping was not done, all cases were screened for the γ -promoter Xmn I site (i.e. –158 C→T mutation) [13].

Results

The relevant characteristics of the β -thal intermedia patients including nationality, molecular and hematological parameters are listed in table 1. Thirteen were Kuwaitis, 4 Jordanians and 1 Iraqi.

The genotypes identified were homozygotes or compound heterozygotes for different combinations of alleles usually associated with silent or mild β -thal. The β -globin mutations are shown in table 1. The mild alleles, IVS-II-1 G→A and IVS-I-6 T→C accounted for 63.9% of all mutations. Other mutations included the silent –101 C→T, mild CD 19 A→G (Hb Malay), –28 (A→C), and CD 8 (–AA). There was a Sicilian $\delta\beta^0$ allele that was iden-

tified by gene mapping; the details of the methodology and specifics of the deletion will be reported separately.

Four families each had homozygosity for IVS-II-1 G→A and IVS-I-6 T→C mutation, i.e. 61.5% of the families studied. One family each had compound heterozygosity for the following combinations: CD 8 (–AA) and –101 (C→T); IVS-I-6 (T→C) and CD 19 (A→G); IVS-II-1 (G→A) and –28 (A→C); IVS-I-110 (G→A) and a Sicilian $\delta\beta^0$ deletion.

Of the 8 Kuwaiti families, 2 brothers (No. 15 and 16) with compound heterozygosity for IVS-I-6 and CD 19 (Hb Malay) had a Thai mother, while of the 4 Jordanian families, 3 (75%) were homozygous for the IVS-I-6 mutation.

The –158 T→C mutation in the G_γ -promoter was found in all the chromosomes carrying the following β -thal mutations: IVS-II-1 G→A, IVS-I-6 T→C and CD 8 (–AA). All others were negative. Only 5 patients had α -thal-2 deletion.

All the patients had a mild phenotype, none was ever on regular transfusion and only one, the IVS-I-110/ $\delta\beta$ compound heterozygote, developed massive splenomegaly with hypersplenism. She required blood transfusion and splenectomy, after which she maintained a reasonable Hb level and was no longer transfused. Hb levels ranged from 7.8 to 11.3 g/dl (mean 9.4 ± 1.1) and Hb F from 12.3 to 98.6% (mean 57.9 ± 37.2).

Discussion

Although Kuwait is a small country in the northeast corner of the Arabian Peninsula, its population of about 2 million is very heterogeneous. It was founded by groups of families who migrated from the Najd region of Saudi Arabia in the 17th or 18th century [17]. However, some of the early settlers included people of Iranian and Iraqi ancestries. Failaka Island, located at the entrance to Kuwait bay, links present-day Kuwait to ancient civilizations [18, 19]. It was part of the Dilmun civilization, which flourished contemporaneously with the Indus Valley and Mesopotamian cultures, with which it had intimate contact, from 3000 to 1200 BC. More recently, it was used as a camp by Alexander the Great and his successors for about a century starting from 300 BC [20]. In more modern times, there has been intimate contact of Arabian Gulf indigenes with people from North and East Africa, the Mediterranean and East Asia. This varied background has had a significant impact on the genetic pool among Kuwaitis.

It is therefore not surprising that there is no discrete segregation of β -thal mutations in the Kuwaiti population. Indeed in a previous study, we found 12 different (mainly Mediterranean, Kurdish and Asian) mutations among our patients [13]. In a study from the United Arab Emirates, whose population is similar to Kuwait's, 35 mutations were reported [21]. This multiplicity of mutations makes it difficult to instate a simple model of screening to facilitate early or prenatal diagnosis. In lieu of this, it is advisable to document the mutations commonly associated with an intermedia phenotype. If the number of such is small, the initial screening can be limited to these. The family of a patient carrying such mutations can then be counseled as to the likely prognosis. Early diagnosis of TI is also important in order to avoid regular transfusions, as for TM, which may lead to significant morbidity from iron overload and other complications [6, 22].

Homozygosity involving the mild β^0 mutation, IVS-II-1 (G→A) and the β^+ mutation, IVS-I-6 (T→C) is associated with the intermedia phenotype in 8 (61.5%) of the 13 families in this study. These 2 along with the β^0 frame-shift codon 8 (–AA), which was found (as a compound heterozygote) in 2 other families (No. 9 and 10), are all Mediterranean mutations known to be associated with a mild phenotype. They were found on chromosomes with the –158 C→T mutation on the G_γ -globin gene promoter. This is responsible for the increased production of Hb F, which in IVS-II-1 and CD 8 homozygotes is about 98% and in IVS-I-6 about 20% [5, 9, 10, 23].

There were 2 brothers (serial No. 15 and 16) who were compound heterozygotes for the IVS-I-6 (C→T) and the CD 19 (A→G) Malay mutations. These boys have a Kuwaiti father and a Thai mother. The CD 19 (A→G) is one of those mutations that activate an alternate splice site and is associated with an intermedia phenotype [24, 25]. –101 (C→T) mutation, found in 1 family, is typically associated with silent β -thal in that heterozygotes have normal hematological parameters (mean corpuscular volume, mean hemoglobin concentration), normal Hb A₂ and Hb F levels. The mutation was originally described from Turkish and Bulgarian families, but subsequently has been seen in Israelis and Italians [5, 26–28]. In the family in this study, the mutation was in compound heterozygosity with another mild mutation, CD 8 (–AA), which is why the patients had such a mild phenotype. The promoter mutation, –28 (A→C), found in compound heterozygosity with IVS-II-1 in 1 patient, is a β^+ mutant originally described in Kurdish Jews [29]. Interestingly, the patient is from Iraq, which has a sizable Kurdish population. In homozygotes, the mutation may be associated with TM, but when

it is inherited along with a mild mutation, as in this patient, the intermedia phenotype prevails.

The only mutation encountered in this study, which is usually associated with a severe β^0 phenotype, is IVS-I-110 (G→A). However, the patient was a compound heterozygote with a $\delta\beta$ allele. The latter is associated with increased Hb F levels, which in this patient was 98%.

Conclusions

Since there are only a limited number of mutations associated with the TI phenotype among Kuwaitis, it is feasible to design a protocol to screen for these first in

every patient presenting with an unclear picture. Strategies still have to be designed to quickly identify other mutations in all patients presenting with a severe thal picture to fully document all mutations encountered in this population.

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