Rare and unexpected mutations among Iranian β-thalassemia patients and prenatal samples discovered by reverse-hybridization and DNA sequencing

We analyzed 70 previously unresolved cases of Iranian β-thalassemia by reverse-hybridization and DNA sequencing. In 60 (86%) of these samples 23 different rare and unexpected β-globin alleles were identified. One of them was a previously unknown transition from AAG (Lys) to TAG (Stop) in codon 95.

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β-thalassemia is one of the most common hereditary disorders in Iran, there being an estimated two million carriers and an extensive spectrum of mutations.[1-3] While peripheral diagnostic laboratories cover the most common β–globin gene mutations known to occur within their respective area, samples remaining untyped are referred to our national reference laboratory for further analysis. Between 1999-2001 we received a total of 70 DNA samples from patients (59 thalassemia trait and 4 thalassemia major subjects) and prenatal cases (4 chorionic villi and 3 amniotic fluid samples), from various geographic areas and ethnic groups within Iran.

As our first approach these samples were screened for a panel of 22 relatively common β-globin mutations using an assay based on polymerase chain reaction (PCR) and reverse-hybridization to oligonucleotide arrays immobilized on test strips.[4] This powerful technique covers more than 80% of known β-globin alleles in Iran in a single amplification and hybridization step, allowing even very small amounts of DNA (e.g. prenatal samples) to be rapidly and comprehensively typed.[5] A total of 65 samples, which remained negative for these prevalent mutations, were subjected to DNA sequencing of the entire β-globin gene including the 5´-untranslated region. The combined results of both techniques revealed a total of 23 different β-globin alleles among our samples (Table 1).

The most common one was IVS I-130 (G-C), which was identified in six subjects from the North of Iran, three subjects from the Southwest, as well as in one DNA of unknown geographical origin. A single homozygous individual showed a severe type of anemia and low hemoglobin (8.7 g/dL), the remaining heterozygotes had a mean Hb A_2 of 5.3% (range: 4.6-6.5), and mean MCV and Hb levels of 61.7 fL (58.0-64.8) and 12.0 g/dL (10.8-13.2), respectively. The second most common mutation was cd 82/83 (-G), which was present in four samples each from Northern and Central Iran, and in one sample from Western Iran. Two of the samples were derived from prenatal cases; the mean Hb, MCV and Hb A_2 level of the remaining seven heterozygous subjects were 12.6 g/dL (10.0-15.1), 62.7 fL (60.0-68.0) and 5.0% (3.9-6.0), respectively. The origin of both IVS I-130 (G-C) and cd 82/83 (-G), as well as a number of other mutations found among our samples, is attributed to neighbors to the North and Northwest of Iran (Turks, Kurds, Azerbaijanies).[6-8] Unlike these, another group of mutations, including the third most common mutation cd 16 (-C) as well as cd 41/42 (-TTCT), is of Asian-Indian or East Asian (Chinese, Japanese) origin.[8] Interestingly and in agreement with our previous observations,[3] frameshift mutation cd 16 (-C) was entirely restricted to chromosomes derived from the Southeast of Iran, suggesting that it should be particularly considered for β-thalassemia testing in that part of the country. A third group of mutations we observed originates from Mediterranean populations; this group included the Portuguese type (TGG-TGA) of cd 15 mutation.[8] This was found in three samples from Western and Southwestern Iran, while the Asian-Indian type (TGG-TAG) of cd 15 mutation was identified in one sample from the Southeast. Among the very rare mutations were two heterozygotes for IVS II-2,3 (+11/-2), which had been discovered earlier in an Iranian family.[9] Very surprisingly, we also identified IVS I-2 (T-C) in an individual from Northern Iran, a mutation which had originally been reported to occur among American Blacks.[10] A previously unknown transition from AAG (Lys) to TAG (Stop) in codon 95, was discovered in a β-thalassemia trait patient from Western Iran presenting with the following hematologic parameters: MCH: 18.7 pg, MCV: 61.1 fL, Hb A: 93.3%, Hb A_2: 5.2%. In a total of 10 cases we could not detect a mutation by our two-step approach, suggesting that the thalassemia phenotype for these patients is caused by genetic defects outside the β-globin gene and 5´-untranslated region.

These results confirm earlier reports from our group as well as other groups on the highly heterogeneous spectrum of β-thalassemia mutations in Iran.[1-3] The large variety of existing alleles and their uneven distribution within the different parts of the country is mainly due to the fact that the Iranian population represents a mixture of various ethnic groups. Refining our knowledge about the distribution of common and rare mutations will further improve carrier detection and prenatal diagnosis to prevent this prevalent disorder.

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Table 1. β-Globin mutations identified by reverse-hybridization and DNA sequencing. Seventy thalassemia patient and prenatal DNA samples were initially tested for 22 common β-globin mutations by reverse-hybridization, and if negative further analyzed by DNA sequencing of the entire β-globin gene and 5´-untranslated region. A total of 23 different mutations discovered by this two-step approach are listed, including number of chromosomes, heterozygous and homozygous cases.

Mutation	*Type*	*Ethnic Origin*	*Heterozygous cases*	*Homozygous cases*	*N. of chromosomes*
IVS I-130 (G-C)	β^0	Turkish	9(1)	1	11
cd 82/83 (-G)	β^0	Azerbaijanian	9(2)		9
cd 16 (-C)	β^0	Asian-Indian	5	1	7
-88 (C-A)	β^+	Kurdish	4		4
-30 (T-A)	β^+	Turkish	4(1)		4
IVS I-128 (T-G)	β^+	Saudi-Arabian	2(1)	1(1)	4
cd 41/42 (-TTCT)	β^0	Chinese	2	1	4
cd 15 (TGG-TGA)	β^0	Portuguese	3		3
cd 25/26 (+T)	β^0	Tunisian	2		2
IVS II-2,3 (+11/-2)	β^0	Iranian	2		2
IVS II-850 (G-T)	β^0	Japanese	2		2
-101 (C-T)	β^+ (silent)	Turkish		1	2
-87 (C-G)	β^+	Mediterranean	1(1)		1
-28 (A-C)	β^+	Kurdish	1		1
Cap+1 (A-C)	β^+	Asian-Indian	1		1
5´UTR +22 (G-A)	β^+	Turkish	1		1
init cd (ATG-ACG)	β^0	Yugoslavian	1		1
cd 15 (TGG-TAG)	β^0	Asian-Indian	1		1
cd 24/25 (-GGT)	β^0	Japanese	1		1
IVS I-2 (T-C)	β^0	American Black	1		1
cd 42/43 (+G)	β^0	Japanese	1		1
cd 95 (AAG-TAG)		Iranian	1		1
IVS II-654 (C-T)	β^+	Chinese	1		1
None	–	–	10		10
Total	–		65	5	75

(1)including 1 prenatal case; (2) including 2 prenatal cases.

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Ursodiol does not prevent hepatic venoocclusive disease associated with Mylotarg therapy

Ursodiol is variably reported to reduce stem cell transplantation (SCT)-associated hepatic venoocclusive disease (VOD). VOD developed in 10 of 85 (12%) patients receiving Mylotarg-based regimens with ursodiol 300 mg *bid* for 21 days beginning on the day prior to cytotoxic therapy – an incidence seen with these regimens when administered without ursodiol.

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Mylotarg (gemtuzumab ozogamicin, CMA-676) is a semisynthetic derivative of calicheamicin linked to a recombinant anti-CD33 monoclonal antibody.[1] In phase I/II studies, Mylotarg has been associated with an approximately 20% incidence of grade 3 or 4 hyperbilirubinemia and raised liver transaminases.[1] VOD has been associated with Mylotarg therapy, both in patients with or without a prior SCT.[1-9] No therapy has been proven to reduce the incidence of SCT-associated VOD.[10] Ursodiol has been variably reported to reduce VOD associated with SCT.[10] We thus assessed the potential efficacy of ursodiol in reducing the incidence and/or severity of Mylotarg-associated VOD in patients with refractory acute myeloid leukemia (AML) or myelodysplastic syndromes (MDS).

The patient's details are presented in Table 1. VOD was diagnosed according to the standard Seattle criteria.[10] All patients gave written informed consent to participation in Institutional Review Board-approved studies. Patients received the Mylotarg-based regimens shown in Table 2. Patients received ursodiol 300 mg *bid* orally starting on the day prior to the first day of Mylotarg or other cytotoxic therapy and continuing for 21 days. No patient

Table 1. Patients' characteristics.

Characteristics	*% of total (n=85)*	*% without VOD (n=75)*	*% with VOD (n=10)*
Diagnosis			
AML	81	85	50
MDS	19	15	50
Prior therapy			
No	60	63	40
Yes	40	37	60
Age > 60 yrs	52	52	55
PS 2-4	75	75	82
Hg < 10 g/dL	81	80	91
WBC > 20×10^9/L	30	32	18
Plts < 100×10^9/L	79	79	82
AHD	40	39	38
Adverse karyotype (–5, –7, 11q23, +8)	22	21	20
Serum bilirubin (mg/dL)			
Baseline	0.8 (0.4-1.7)	0.8 (0.4-1.4)	0.8 (0.4-1.7)
Maximum elevation	1.9 (0.4-9.6)	1.5 (0.4-1.3)	4.5 (2.6-9.6)
Days to maximum elevation	5 (2-25)	4 (2-8)	15 (4-25)
SGPT (μ/L)			
Baseline	36 (12-239)	36 (12-239)	39 (12-112)
Maximum elevation	118 (12-3966)	106 (12-3966)	280 (52-2370)
Days to maximum elevation	13 (5-27)	13 (5-27)	13 (7-23)