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Full Length Research Paper

Rare and unexpected beta thalassemic mutations in Qazvin province of Iran

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About 13 beta-globin mutations encompass 70 - 90% of mutation spectrum in Iran. These mutations are called common beta-globin mutations. The rest are rare or unknown mutations. The objective of this study was to identify and describe rare or unknown beta-globin mutations in Qazvin province. EDTA-containing venous blood samples were collected from 100 patients with transfusion-dependent beta-thalassemia from the department of pediatrics of Qods hospital of Qazvin. Screening for causal mutations was carried out on DNA isolated from WBC´s of the patients by using Amplification Refractory System (ARMS) technique. 14.1% of alleles which were not discovered by ARMS, were uncovered by direct sequencing that include 9 different rare mutations. Thirty-seven combinations of alleles (genotypes) were recognized in all affected patients. The frequency of mutations of nt-30, IVS I-6, Cd5, IVS II-745, 5´ UTR, Cd15, Cd39, IVS I-130, Cd24, Cd74/75, HbS and Hb Monroe were about 1% or less. We have revealed and described the existence of 9 rare mutations from Qazvin, two of which (Cd74/75 and Hb Monroe) are the first reported in Iran.

Key words: Rare thalassemia mutations, beta globin gene, Qazvin, direct sequencing.

INTRODUCTION

Beta thalassemia is considered as the most common autosomal single-gene disorder world wide. it can be found in more than 60 countries with a carrier (heterozygote) population of up to 150 - 200 million people or 4.5% of the world population and at least 300000 lethally affected homozygotes are born annually (Kawthalklar, 2006 ; Old et al., 1990).

Iran, with more than 18000 affected individuals, represents one of the areas in the world with an unusually high prevalence of beta thalassemia. Provinces around the Persian Gulf and the Caspian Sea with a gene frequency of more than 10% constitute the thalassemia major zones in Iran (Najmabadi et al., 2003; Derakhshandeh et al., 2007).

The disease results from one or more of about 200 different mutations in the beta globin gene (HBB). So there are numerous gene mutations responsible for beta thalassemia. This disease can be induced in Iran by at least 43 different mutations. Each province of Iran has its own characteristic spectrum of mutations, with a handful of frequent mutations and several rare ones. Searching for and identifying rare or new mutations is a constant priority in population screening, genetic counseling and prenatal diagnosis (PND) of thalassemia. About 13 beta-globin mutations encompass 70 - 90% of mutation spectrum in Iran. These mutations are called common beta-globin mutations (Roudkanar et al., 2003). The rest are rare or unknown mutations. In our past study we descrybed the spectrum of thalassemia mutations in Qazvin province (Sarookhani et al., 2009). The objective of current study is the analysis of rare or unexpected beta-globin mutations in Qazvin.

MATERIALS AND METHODS

In an analytical-descriptive study venous blood samples (always prior to transfusion) were collected in EDTA-containing tubes from 100 patients (9 related families and 91 unrelated) with transfusion-dependent beta thalassemia major from the Department of Pediatrics of Qods Hospital of Qazvin.

The age, sex, duration and start of transfusion and consanguinity (familial marriage) between parents were recorded by reviewing

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Table 1. Oligonucleotide primers used for mutation detection by direct sequencing.

5´-TTGAGGATTCGGTCACGGTCTTCT -3´	Sequencing primer Sense
5´-TTTTCCCTTACACCCTCCAGTCAC -3´	Sequencing primer Anti sense
5´- CTTAGGCTGCTGGTGGTCTACC- 3´	Sequencing primer F
5´- AGCACTTTCTTGCCATGAGCC- 3´	Sequencing primer R

Table 2. Degree of incidence of various mutations in beta thalassemia major patients of Qazvin province.

incidence	mutation	No. and % of alleles	Frequency (%)
HIGH	Ivs 2/1	57 (31.3%)	59.3%
	Ivs 1/110	35 (19.2%)	
	Fsc 8/9	16 (8.8%)	
MODERATE	Codon 30	9 (4.95%)	25.85%
	Codon 44	11 (6.05%)	
	Ivs 1/5	10 (5.5%)	
	Fsc 36/37	9 (4.95%)	
	Ivs 1/1	8 (4.4%)	
LOW	nt -30	4 (2.2%)	12.1%
	Ivs 1/6	3 (1.65%)	
	Codon 5	3 (1.65%)	
	Ivs 2/745	2 (1.1%)	
	5 UTR	2 (1.1%)	
	Codon 15	2 (1.1%)	
	Codon 39	2 (1.1%)	
	Ivs 1/130	2 (1.1%)	
	No mutation	2 (1.1%)	
RARE	Codon 24	1 (0.55%)	2.75%
	Codon 74/75	1 (0.55%)	
	Hbs	1 (0.55%)	
	Hb Monroe	2 (1.1%)	
SUM	20	182	100%

their files after obtaining their (or parents) permission.

DNA extraction from samples and Amplification Refractory System (ARMS) technique for screening of common thalassemia mutations were performed as described before (Sarookhani et al., 2009; Najmabadi et al., 2001).

A total of 13 samples, which remained negative for common or prevalent mutations, were subjected to DNA sequencing of the beta-globin gene. To identify the rare beta thalassemia alleles of the study, two region of beta globin of the samples were amplified by two sets of primers (sense, anti sense, F primer and R primer) (Table 1) designed for initial site, exon I, intron I as well as exon II and intron II of the gene (Mirasena et al., 2007). These primers were synthesized in ATG Copenhagen, Denmark. The resulting segments (850 and 500 bp) and nucleotides respectively, contain most of the known mutant sites specific for the Mediterranean population.

Following amplification, the PCR products were purified, using an ABI purification kit. The purified samples were sequenced using the dideoxy termination procedure of Sanger (Sanger, 1997) by an automated sequencer analyzer (ABI-3730XL Capillary, Applied Biosystem, USA) as per the manufacture's instructions. Comparisons of sequencing results with normal beta-globin gene sequence were performed using Gene runner software.

RESULTS

The various mutations in unrelated transfusion dependent beta thalassemias (91 patients) of Qazvin province according their degree of incidences are given in Table 2.

Based on these results, the frequency of different kinds of low frequent and rare mutations was 14.85%. Mutations of nt-30, IVS I-6, Cd5, IVS II-745, 5 UTR, Cd15, Cd39, IVS I-130, Cd24, Cd74/75 HbS and Hb Monroe were observed rarely (about 1% or less) in this region. One patient (2 alleles) remained unknown. Most of these rare mutations (alleles) in patients, were co-inherited (in combination) with frequent mutations (combined heterozygote conditions). Thirty seven different combinations

Table 3. Genotype frequencies of thalassemia major population (all relatives and non-relatives) of Qazvin province.

No.	Genotype	Frequency (%)
1	Ivs 2/1, Ivs 2/1	18
2	Ivs 1/110, Ivs 1/110	10
3	Fsc 8/9, Fsc 8/9	5
4	Codon 44, Codon 44	1
5	Ivs 2/1 , Ivs 1/110	13
6	Codon 30 , Ivs 2/1	6
7	Ivs 1/1 , Ivs 1/110	4
8	Ivs 1/5 , Ivs 2/1	4
9	Codon 44 , Ivs 2/1	4
10	Ivs 2/1 , Codon 5	1
11	Codon 44 , Codon 30	1
12	Codon 5 , Codon 44	1
13	Ivs 1/110 , Codon 44	1
14	vs 1/5 , Codon 44	2
15	Ivs 2/745 , Ivs 2/1	1
16	Ivs 1/1 , Ivs 2/1	1
17	Codon 44 , Ivs 2/745	1
18	Ivs 1/6 , Ivs 1/5	1
19	Fsc 8/9 , Ivs 2/1	1
20	Codon 30, Codon 30	1
21	Fsc 8/9 , 5 UTR	1
22	Fsc 36/37, Fsc 36/37	5
23	Fsc 8/9 , Codon30	1
24	Ivs 1/1, Ivs 1/1	1
25	Ivs 1/1 , Ivs 1/130	1
26	Codon 24 , Codon 30	2
27	Fsc 8/9 , Hbs	1
28	Codon 39, Codon 39	1
29	Codon 15 , Codon 15	1
30	Ivs 1/5 , Ivs 1/5	1
31	5 UTR , Ivs1/5	1
32	Ivs 1/6 , Ivs 1/6	1
33	Codon 5,Codon 74/75	1
34	Fsc 8/9 , Ivs 1/130	1
35	Fsc 8/9 , Fsc 36/37	1
36	nt -30 , nt -30	2
37	No mutation	1
SUM		100

of alleles (genotypes) were recognized (Table 3). Sequencing chromatograms of some rare mutations are shown in Figure 1.

DISCUSSION

Qazvin is a small province with population of about 1.2 million, suited in the north of Iran between Tehran, Gilan, Mazandaran, Zanjan and Hamedan provinces. It is worth mentioning that due to bulky ethnic (genetic) admixture and relatively rich genetic pool in such small province like Qazvin, the distribution of low frequent or rare mutations are high. Two of these mutations were unique to Qazvin province and have not been discovered in any other provinces of Iran. Several interesting descriptions come from the analysis of different kinds of rare mutations of our results, discussed below.

Nucleotide -30(nt -30) (T to A)) was reported in Iran as a rare transcriptional mutation with a frequency of 0.15% (Yavarian, 2005). This mutation has Turkish-Iranian origin (Najmabadi et al., 2002) with β+ phenotype and thalassemia intermedia or even without presentations (Çiğdem, 2002; Oner et al., 2001). Both index cases in homozygous form of our study (Figure 1A) have Turkish origin and thalassemia intermedia phenotypes.

5 ´UTR (+22) (G to A) cap site mutation has been reported very rarely (0.04%) in Iran (Yavarian, 2005; Najmabadi et al., 2002) in Turkish ethnic groups. Its presentations is β+ thalassemia intermedia in homozygous form (Çiğdem, 2002). In our study both alleles of this mutation have been inherited as compound heterozygote (5´ UTR / IVS I-5 and 5´ UTR / FSC 8 - 9 genotypes).

Codon 15 (TGG to TGA or stop codon) as a rare nonsense mutation has been reported in Iran by Najmabadi et al. (3 alleles of Portuguese and 1 allele of Asian-Indian (TGG to TAG) types) (Najmabadi et al., 2002). It has 0.11% overall frequency in Iran (Yavarian, 2005). In present study this mutation was detected in homozygous form (Figure 1B) of Portuguese type in a Turkish patient.

IVS I-130 mutation (G to C) with a Turkish-Iranian origin was reported as a most common of rare mutations in Iran by Najmabadi et al. (11 allele), Rahim et al. (1 allele) and Roudkanar et al. (4 allele) (Najmabadi et al., 2002; Rahim et al., 2007; Roudkanar et al., 2003). In present study this mutation was observed in 2 cases with Turkish origin in compound heterozygote forms (IVS I-1 / IVS I-130 and FSC 8 - 9 / IVS I-130 genotypes). Yavarian has reported a frequency of 0.41% for this mutation in Iran (Yavarian, 2005).

Codon24 (GGT to -GT) mutation was reported very rarely (0.04%) in Iran (Yavarian, 2005, Najmabadi et al., 2002). In present study only one allele of this mutation in combination with Codon30 mutation (Codon 24/ Codon 30 genotype) was detected.

HbS (GAG to GTG; Glu to Val) is a common hemoglobinopathy in south of Iran (Rahimi et al., 2006). It can be co-inherited with different kinds of thalassemia mutations (sickle-thalassemia or double heterozygote conditions). To the best of our knowledge it has not been reported in north of Iran in literature reviews. In present study it was detected in the form of FSC 8 - 9/ HbS genotype producing severe phenotype of beta thalassemia major. Rahimi et al. was reported such genotype in south of Iran (Rahimi et al., 2006).

Hb Monroe (beta 30, G to C or IVS I(-1)) results from a splice site point mutation in the last nucleotide of beta-globin

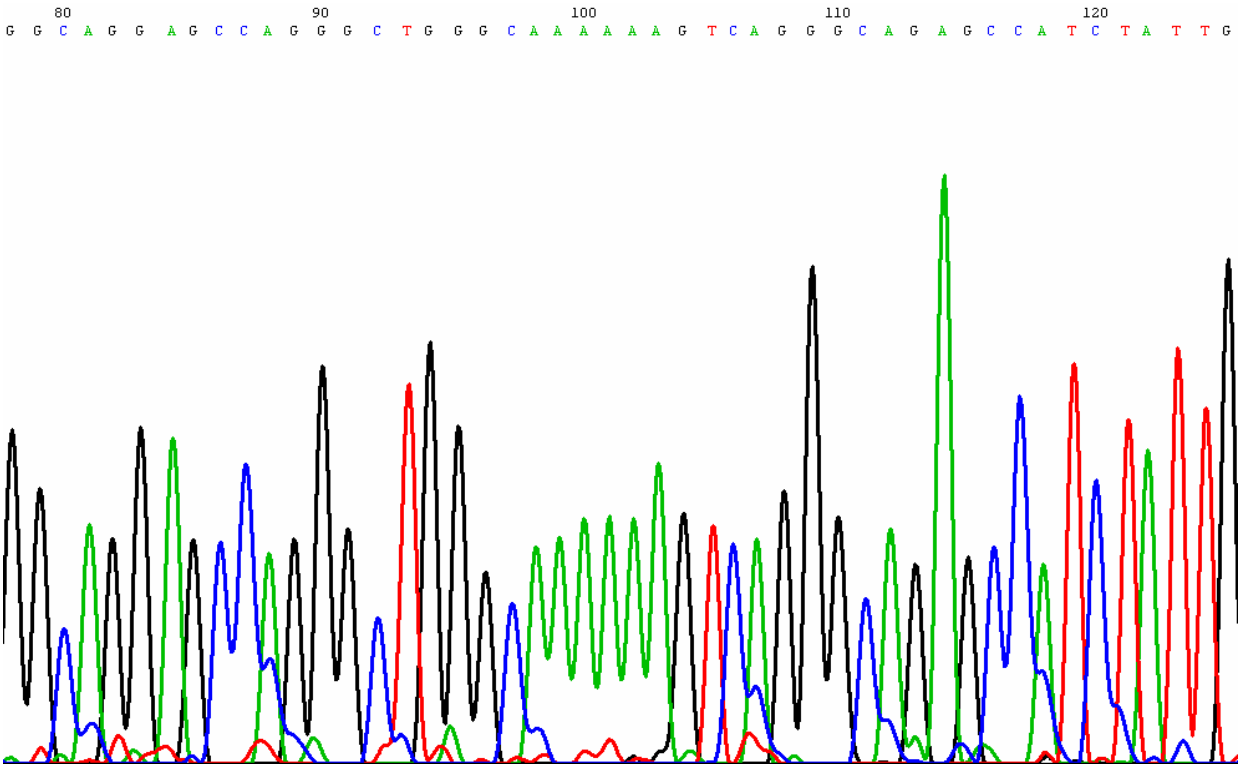

Figure 1A. Sequence obtained from automated DNA sequencing of some uncharacterized (by ARMS technique) thalasemia major samples in Qazvin province. **nt-30 mutation:** There is a transition of T to A in promoter region (Transcription defect), homozygote case.

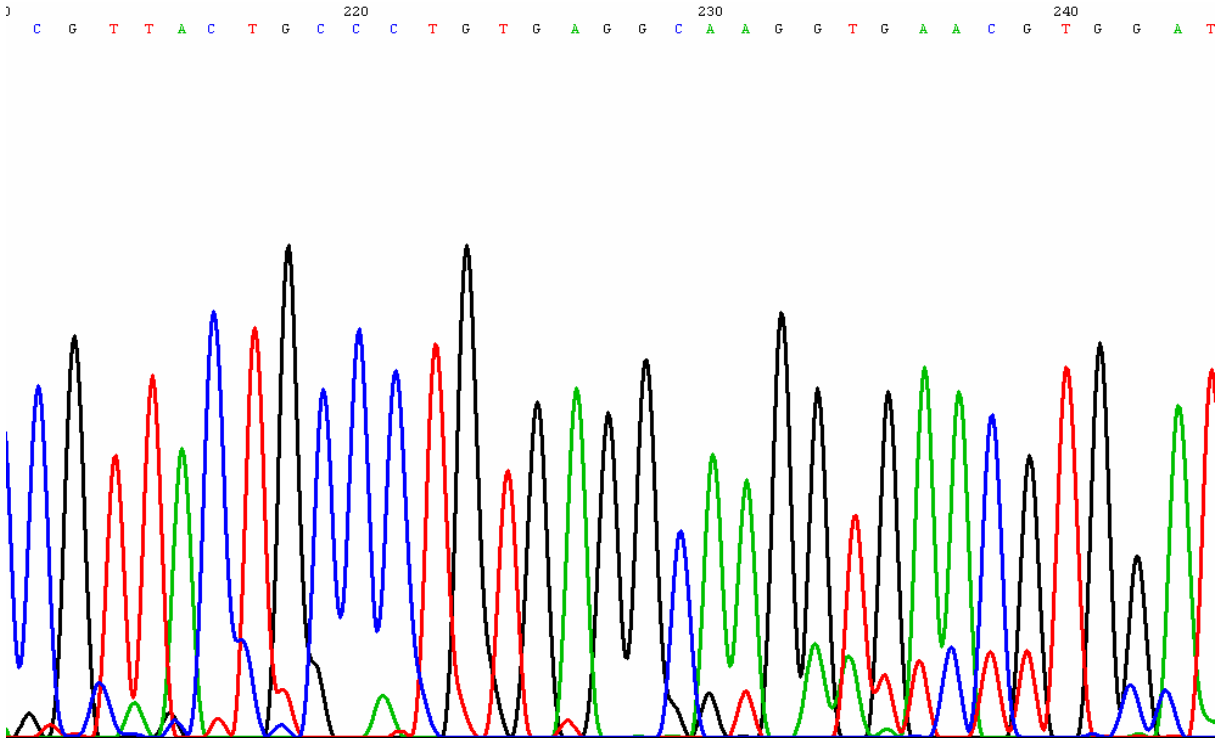

Figure 1B. Sequence obtained from automated DNA sequencing of some uncharacterized (by ARMS technique) thalasemia major samples in Qazvin province. **Codon 15 mutation:** There is a substitution of G to A in codon 15, changing Trp codon (TGG) to stop codon (TGA), homozygote case.

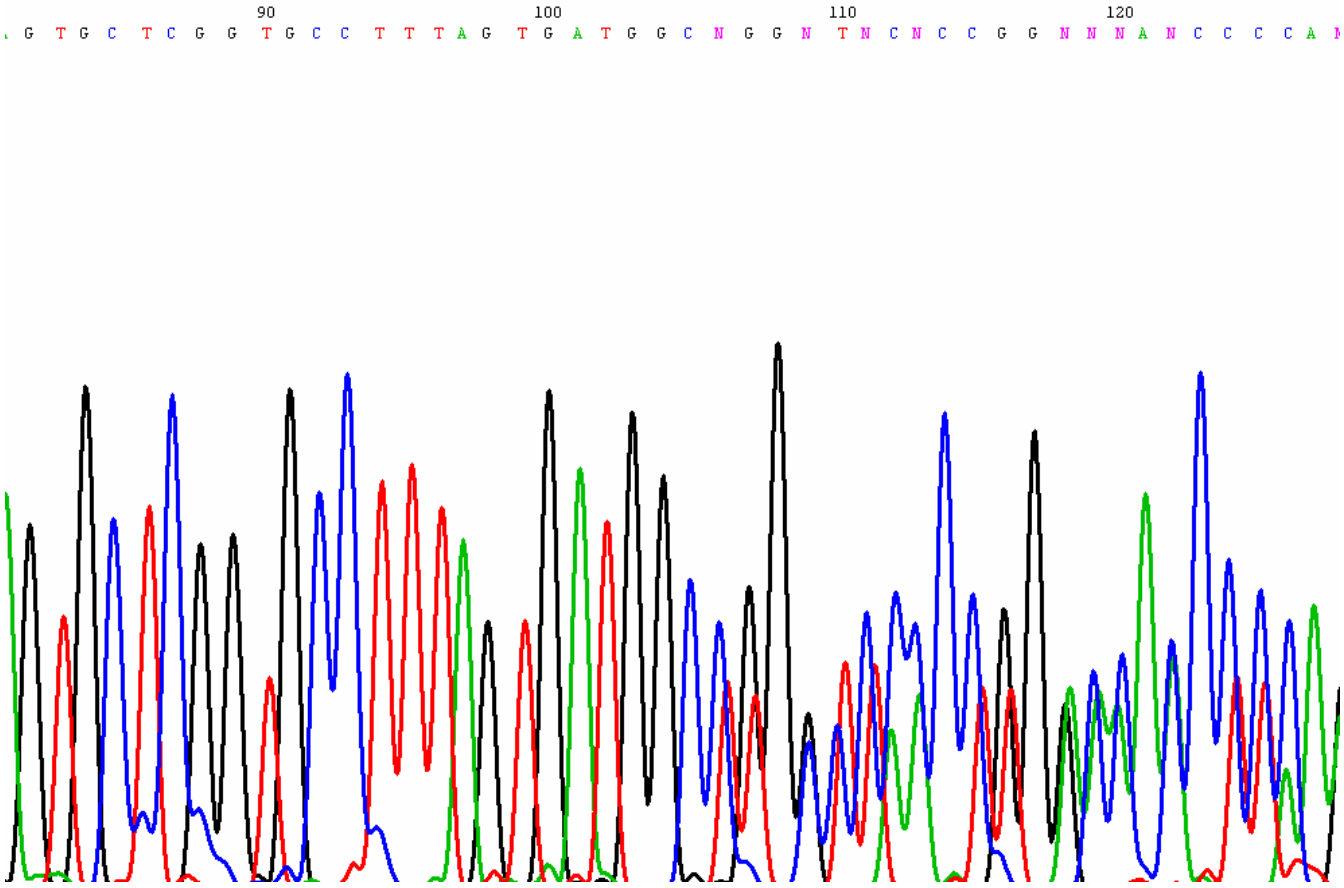

Figure 1C. Sequence obtained from automated DNA sequencing of some uncharacterized (by ARMS technique) thalasemia major samples in Qazvin province. **Codon 74/75mutation:** There is a deletion of C at last nucleotide of codon 74 (GGC CTG to GG-CTG), producing frame shift of next codons (heterozygote case).

exon1 which is also the penultimate nucleotide of codon 30 of the beta-globin peptide (AGG to ACG ; Arg to Thr). After the original reports in 1989, Hb Monroe has been reported in low frequency, mostly in compound heterozygosity with other beta globin mutations (Agarwal et al., 2007). We have already reported this mutation in Iran (Sarookhani et al., 2009) which co-inherited with IVS II-1 (Figure 1D). Before this, there is no report of such mutation and genotype in Iran (Roudkanar et al., 2003; Yavarian, 2005; Najmabadi et al., 2002; Najmabadi et al., 2001).

Codon74/75 (GGC CTG to GG- CTA) is a rare frame shift mutation in the world with βº phenotype in Turkish ethnic groups (Çiğdem, 2002). It was detected in compound heterozygote form (Codon 5/ Codon 74/75) in one allele of the present study (Figure 1C). A literature search has shown that this is the first report of this mutation from Iran and has not been reported by any author in their reviews on thalassemia mutation in Iran.

In spite of having thalassemia major phenotype, one patient (2 alleles) detected as "no mutation". This case need to be subjected to further detailed investigations which include analysis of the beta-globin gene that could include defects in beta-globin Locus Control Region (LCR).

Conclusion

Heterogeneity of beta-thalassemia mutations in a quite small area may be regarded as footprints of many population movements. The present study indicates that the frequency of mutations at Qazvin province is different than the mutation frequency profile given for Iran. Several combinations of alleles detected here may be considered important in terms of understanding of molecular defects causing beta-thalassemia. We also identified different kinds of rare and new mutations in the population of the Qazvin province, two of which (Cd74/75 and Hb Monroe) as reported previously (Sarookhani et al., 2009) are the first reported in Iran.

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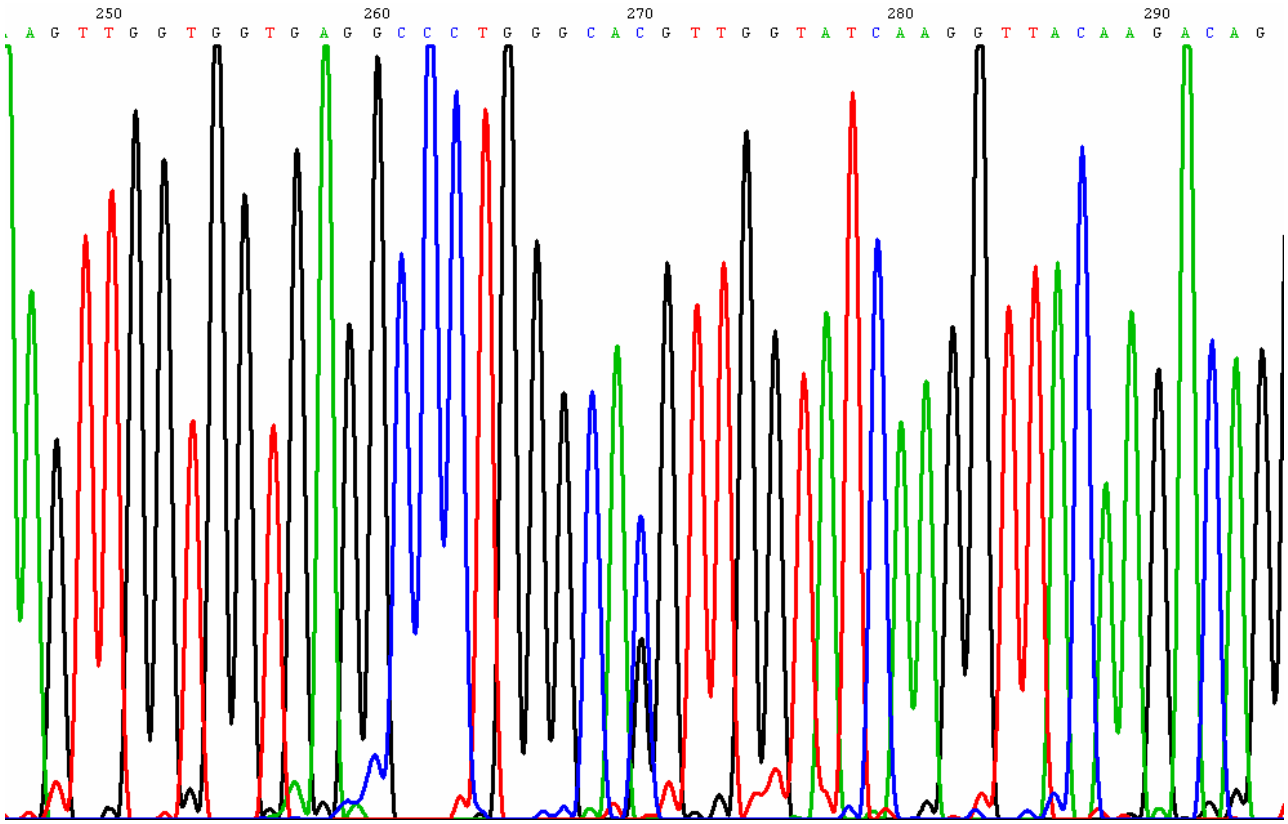

Figure 1D. Sequence obtained from automated DNA sequencing of some uncharacterized (by ARMS technique) thalasemia major samples in Qazvin province. **Hb Monroe mutation:** There is a substitution of G to C in the last nucleotide of beta-globin exon1 which is also the penultimate nucleotide of codon 30 of the beta-globin peptide (AGG to ACG; so Arg to Thr) (heterozygote case).

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REFERRENCES

Agarwal N, Kutlar F, Mariluz P, Henshaw M, Ching NO, Gaikwad MN, Reading S, Bailey L, Kutlar A, Prchal JT (2007) Missence mutation of the last nucleotide of exon 1 (G to C) of beta-globin gene not only leads to undetectable mutant peptide and transcript but also interferes with the expression of wild allele. Haematologica, 92(12): 1715-1716.

Çiğdem A (2002). The Frequency and Distribution Pattern of ß-Thalassemia Mutations in Turkey. Turk. J. Hematol. 19(2): 309-315.

Derakhshandeh-Peykar P, Akhavan-Niaki H, Tamaddoni A, Ghawidel-Parsa S, Naieni KH, Rahmani M, Babrzadeh F, Dilmaghani-Zadeh M, Farhud DD (2007). Distribution of β-thalassemia mutations in the northern provinces of Iran. Hemoglobin, 31(3): 351-356.

Kawthalklar SM (2006). In: Anemia due to red cell destruction. Essentials of haematology. 1st ed. India. Jaypee brothers medical publishers Co; pp. 140-147.

Mirasena S, Shimbhu D, Sanguansermseri M, Sanguansermseri T. (2007). The spectrum of β-thalassemia mutations in phitsanulok province: Development of multiplex ARMS for mutation Detection. Naresuan University Journal, 15(1): 43-53.

Najmabadi H, Karimi-Nejad R, Sahebjam S, Pourfarzad F, Teimourian S, Sahebjam F, Amirizadeh N, Karimi-Nejad MH (2001). The β-thalassemia mutation spectrum in the Iranian population. Hemoglobin, 25(3): 285-296.

Najmabadi H, Neishabury M, Sahebjam F, Kahrizi K, Shafaghati Y, Nikzat N, Jalalvand M, Aminy F, Bany Hashemi S, Moghimi B, Noorian AR Jannati A Mohammadi M, Javan K (2003). The Iranian Human Mutation Gene Bank: A data and sample resource for worldwide collaborative genetics research. Hum. Mutat. 21: 146-150.

Najmabadi H, Pourfathollah AA, Neishabury M, Sahebjam F, Krugluger W, Oberkanins C (2002). Rare and unexpected mutations among Iranian b-thalassemia patients and prenatal samples discovered by reverse-hybridization and DNA sequencing. Haematologica. 87: 1113-1114.

Najmabadi H, Teimourian SH, Khatibi T, Neishabury M, Poufrafzad F, Jalil Negad S, Azad M, Oberkanins CH, Krugluger W (2001). Comparison of Amplification Refractory System (ARMS) and Reverse Hybridization for the detection of the 10 most common Beta-Thalassemia mutations within the Iranian Population. Arch. Iran Med. 4(4):165-170

Old JM, Varawalla NY, Weatherall DJ (1990). Rapid detection and prenatal diagnosis of b-thalassemia: studies in Indian and Cypriot populations in the U.K. Lancet, 336: 834-837.

Oner AF, Ozer R, Uner A, Arslan S, Gumruk F (2001). Beta-Thalassemia Mutations in the East of Turkey. Turk. J. Hematol. 18(4): 239-241.

Rahimi F, keikhaei B, Aberumand M (2007). PrenatalDiagnosis (PND) of beta thalessemia in the Khuzestan province Iran. J. Clin. Diagn. Res. 1: 454-459.

Rahimi Z, Vaisi Raygani A, Merat M, Haghshenass N, Gerard RL, Nagel RK (2006). Thalassemia mutations in southern Iran. Iran J. Med. Sci. 31(2): 70-73

Roudkanar MH, Najmabadi H, Derakhshandeh P, Farhud DD (2003). Detection of rare and unknown mutations in β-thalassemia traits in

Iran. Iran. J. Public Health, 32(1): 11-14.
Sanger F (1997). DNA sequencing with chain termination inhibitors. Proc. Natl. Acad. Sci. 74: 5463-5467.
Sarookhani MR, Ahmadi MH, Amirizadeh N (2009). Molecular Spectrum of Beta-Globin Mutations in Transfusion-Dependent Patients with Thalassemia in Qazvin Province, Iran. Iran J. Med. Sci. 34(1): 17-22.
Yavarian M (2005). Hemoglobinopathies in Iran; molecular spectrum, prevention and treatment. Leiden university publications. The Netherland. (Available on: http://hdl.handle.net/1887/3728.)