Beta-thalassemia mutations in Denizli province of Turkey

Sanem YILDIZ[1], Ayfer ATALAY[2], Hüseyin BAĞCI[3], Erol Ömer ATALAY[1]

[1] Department of Biophysics, Pamukkale University School of Medicine,
[2] Pamukkale University Research Center for Genetic Engineering and Biotechnology,
[3] Department of Medical Biology, Pamukkale University School of Medicine, Denizli, TURKEY

Turk J Haematol 2005;22(1): 19-23

Received: *15.06.2004* ***Accepted:*** *14.09.2004*

ABSTRACT

Beta-thalassemia is one of the most common genetic disorders in Turkey as well as in several other Mediterranean countries presenting microcytosis and hemolytic anemia. The city of Denizli is located in the inner part of the Aegean geographical region of Turkey. The beta-thalassemia incidence in Denizli province is in between 2.6-3.7% reported by different researchers. According to our results; the IVS-1/nt-110 (G>A) is the most frequent mutation type in our province the same as other geographical regions of Turkey. Here we report also two HbD-Los Angeles/beta-thalassemia combinations, which are HbD-Los Angeles/codon 39 (C>T) and HbD-Los Angeles/IVS-1/nt-1 (G>A), respectively. In conclusion, our preliminary results show the heterogeneity of the beta-thalassemia mutations in the province of Denizli.

Key Words: Beta-thalassemia, Mutation, Turkey.

ÖZET

Denizli bölgesinde beta-talasemi mutasyonları

Beta-talasemi, özellikle Akdeniz ülkelerinde ve Türkiye'de görülen, mikrositoz ve hemolitik anemi tablosu ortaya koyan en yaygın kalıtsal bozukluklardan bir tanesidir. Denizli ili, Ege Bölgemizin iç kesiminde yer almaktadır. Denizli yöresindeki beta-talasemi dağılımı, çeşitli araştırıcıların verdikleri sonuçlara göre %2.6-3.7 arasındadır. Elde ettiğimiz sonuçlarda bölgemizde en sık görülen beta-talasemi mutasyonu IVS-1/nt-110 (G>A) olarak saptanmaktadır. Ayrıca bu çalışmamızda iki tane HbD-Los Angeles/beta-talasemi kombinasyonu rapor edilmektedir. Bu kombinasyonlar sırası ile HbD-Los Angeles/codon 39 (C>T) and HbD-Los Angeles/IVS-1/nt-1 (G>A) şeklinde tanımlanmıştır. Özet olarak çalışmamızda elde edilen veriler, Denizli yöresindeki beta-talasemi mutasyonlarının heterojen yapıda olduğunu göstermektedir.

Anahtar Kelimeler: Beta-talasemi, Mutasyon, Denizli, Türkiye.

INTRODUCTION

Beta-thalassemia is one of the most common genetic disorders in Turkey as well as in several other Mediterranean countries presenting microsytosis and hemolytic anemia[1-3]. Research on beta-thalassemia and other hemoglobin disorders in Turkey had been initiated by Aksoy et al in 1941[4]. In 1971, the prevalence of beta-thalassemia in Turkey was reported as 2%[1]. There are many research studies on the prevalance of beta-thalassemia regarding to the distribution on country scale in Turkey[4-9]. These research studies enlarged under the development of molecular techniques used for either detection and/or its prenatal diagnosis[10-15]. Denizli is located in inner part of Aegean region of Turkey. The beta-thalassemia incidence in Denizli is in between 2.6-3.7% reported by different researchers[16-18]. Premarital screening is applied in Denizli province by the Turkish Ministry of Health Thalassemia Laboratory since 1985. Pamukkale University Research Center for Genetic Engineering and Biotechnology (PAMGEN) Molecular Diagnosis Laboratory has been established by the year of 2003 and initiated the molecular diagnosis of beta-thalassemias and abnormal hemoglobins. The total number of the individuals who applied for molecular diagnosis to our Center is 260 by the date of May 2004. Here we report the preliminary data for beta-thalassemia mutations of these individuals who are unrelated with each other, observed in Denizli province of Turkey.

MATERIALS and METHODS

The total number of individuals is 260 tested for hemoglobin disorders for the year of 2004. Some of them were couples at risk which were informed during premarital screening program at Turkish Ministry of Health, Denizli Thalassemia Laboratory. Since some individuals are relatives, their data was excluded for this manuscript. On the other hand, abnormal hemoglobins except HbDLos Angeles/beta-thalassemia combinations were also excluded from the data. The number of individuals which are unrelated with each other is 53. Written informed consent was obtained from all individuals in the study. Alkaline and acid hemoglobin electrophoresis was performed with agarose based kits of Helena Biosciences. DNA was isolated with standard phenol-chloroform extraction, ethanol precipitation procedure and mutation analysis for the β-globin gene was done with β-Globin Strip assay (ViennaLab cat.no. 4-120, Austria)[19]. For the molecular detection of HbD variants at β-globin codon 121, genomic DNA was amplified the primers of PAM117 (5'-CAA TGT ATC ATG CCT CTT TGC ACC-3') and PAM118 (5'-GAG TCA AGG CTG AGA GAT GCA GGA-3') and digested with EcoRI[20]. PCR product is 861 bp in length and restriction digests examined by 2% agarose gel electrophoresis. In case of mutation in codon 121, the EcoRI restriction site on codon 121 is being lost. For normal β-globin gene, two fragments, 552 bp and 309 bp in length are observed after EcoRI digestion of the PCR product. The samples diagnosed as HbD-Los Angeles by EcoRI-SNP analysis were confirmed also with DNA sequencing. DNA sequencing was done by radioactively labelled manual dideoxy termination procedure. DNA sequencing for HbD-Los Angeles case was done in Çukurova University Medical Faculty Department of Biochemistry.

RESULTS

Table 1 shows the results of molecular diagnosis for the 55 chromosomes from 53 individuals with beta-thalassemia trait, analyzed with 20 different beta-thalassemia mutations. Approximately ninety five percent of the chromosomes were identified with these 20 mutations. The most frequent beta-thalassemic mutation was found to be IVS-1/nt-110 (G>A). Two individuals were HbD-Los Angeles/beta-thalassemia combinations. Both of them were HbD-Los Angeles/beta-thalassemia combinations, carrying the mutations HbD-Los Angeles/codon 39 (C>T) and HbD-Los Angeles/IVS-1/nt-1 (G>A), respectively.

Table 1. Frequencies of beta-thalassemia mutations in Denizli province of Turkey

Mutation type	No. of chromosomes
IVS-1/nt-110 (G>A)	20
IVS-1/nt-1 (G>A)	9
IVS-1/nt-6 (T>C)	4
IVS-2/nt-745 (C>G)	4
IVS-1/nt-5 (G>C)	1
Codon 8 (-AA)	2
Codon 39 (C>T)	5
-87	2
IVS-2/nt-1 (G>A)	2
-30 (C>G)	NF
Codon 5 (-CT)	NF
Codon 6 (-A)	NF
Codon 8/9 (+G)	1
Codon 22 (7 bp del)	NF
Codon 30 (G>C)	NF
IVS-1/nt-2 (T>A)	NF
IVS-1/nt-116 (T>G)	NF
IVS-1/nt-25 (25 bp del)	NF
Codon 36/37 (-T)	NF
Codon 44 (-C)	NF
HbD-Los Angeles	2
Unknown	3
Total	55

NF: Not found.

The presence of HbD-Los Angeles as carrier state was confirmed by both with EcoRI SNP analysis and DNA sequencing.

DISCUSSION

The preliminary results are the first published data from Denizli province of Turkey concerning the beta-thalassemic mutations. Table 1 summarizes the data obtained in the base of mutations and Table 2 summarizes the comparison of the data related with Aegean Region studies present in the literature[11,21]. Data is consistent, in some respects, with the national and regional distributions of the beta-thalassemic mutations, for the incidence of IVS-1/nt-110 (G>A) mutation as the most common mutation type. Here we report for the first time, up to our knowledge, two HbD-Los Angeles/beta-thalassemia combinations from Denizli province of Turkey. We would like to emphasize that the presence of HbD-Los Angeles and other abnormal hemoglobin types is greater than expected according to our unpublished results.

Our data is more closely related with Gülesken et al. Tadmouri et al did not report for the IVS-1/nt-1 (G>A), codon 39 (C>T) and –87 mutations for Aegean/Mediterranean part of Turkey[11,21]. This is probably due to the relatively small sample size and the large covering area of Aegean/Mediteranean region. Similar situation appears also with the publication of Atalay et al[9]. Since Atalay et al show the mutation data as Western part of Turkey, including Aegean, Marmara and inner part of Anatolia, therefore we do not include these data into the comparison table. In case of the Gülesken et al results, they did not report IVS-1/nt-5 (G>C) and codon 8 (-AA) mutations. We identified IVS-1/nt-5 (G>C) and codon 8 (-AA) mutations as 1.8% and 3.6% respectively. On the other hand, we also report the presence of codon 8/9 (+G) mutation in Denizli province of Turkey. We could not identify 5.5% of our thalassemia trait cases with these 20 Mediterranean type of mutations. This finding also shows the heterogenousity of the beta-thalassemia mutations in our province.

In conclusion; we report for the first time the beta-thalassemia mutational data related with the Denizli province of Turkey. Our data show the complexity of the beta-thalassemia mutations in our area due to the historical aspects and geographical location of Denizli province in Anatolian basin. The research and screening studies on the individual regions will show much more detailed mutation profiles contributing the knowledge of national mutation databases leading to the prenatal diagnostic approaches for the thalassemia control programs in Turkey.

Table 2. Beta-thalassemia mutations in Denizli Province of Turkey in comparison with other publications on Aegean region

	Number of beta-thalassemic chromosomes		
Mutation type	**Denizli province**	**Gülesken et al[21] (Aegean Region)**	**Tadmouri et al[10] (A/M* Region)**
IVS-1/nt-110 (G>A)	36.4%	44.1%	56.7%
IVS-1/nt-1 (G>A)	16.4%	28.2%	-
IVS-1/nt-6 (T>C)	7.3%	13.3%	13.3%
IVS-2/nt-745 (C>G)	7.3%	9.3%	-
IVS-1/nt-5 (G>C)	1.8%	-	6.7%
Codon 39 (C>T)	9.1%	2.4%	-
-87	3.6%	NF	-
Codon 8 (-AA)	3.6%	-	6.7%
IVS-2/nt-1 (G>A)	3.6%	2.7%	3.3%
-30 (C>G)	NF	-	6.7%
Codon 5 (-CT)	NF	-	3.3%
Codon 6 (-A)	NF	NF	-
Codon 8/9 (+G)	1.8%	-	-
Codon 22 (7 bp del)	NF	-	-
Codon 30 (G>C)	NF	-	-
IVS-1/nt-2 (T>A)	NF	-	-
IVS-1/nt-116 (T>G)	NF	-	-
IVS-1/nt-25 (25 bp del)	NF	-	-
Codon 36/37 (-T)	NF	-	-
Codon 44 (-C)	NF	-	-
HbD-Los Angeles	3.6%	-	-
Unknown	5.5%	-	3.3%
Total	55** (100%)	150** (100%)	30** (100%)

* A/M: Aegean/mediterranean region.
** Total number of chromosomes.
NF: Not found.

REFERENCES

1. Çavdar AO, Arcasoy A. The incidence of ß-thalassemia and abnormal hemoglobins in Turkey. Acta Hematol 1971;45:312-8.
2. Kazazian HH, Boehm C. Molecular basis and prenatal diagnosis of β-thalassemia. Blood 1988;72:1107-16.
3. Cao A, Gossens M, Pirastu M. β-thalassemia mutations in Mediterranean populations. Br J Hematol 1989;71:309-12.
4. Aksoy M. The history of β-thalassemia in Turkey. Turk J Pediat 1991;33:195-7.
5. Akar N, Çavdar AO, Dessi E, Loi A, Pirastu M, Cao A. Beta thalassaemia mutations in the Turkish population. J Med Genet 1987;24:378-9.
6. Öner R, Altay Ç, Gürgey A, Aksoy M, Kılınç Y, Stoming TA, Reese AL, Kutlar A, Kutlar F, Huisman TH. Beta-thalassemia in Turkey. Hemoglobin 1990; 14:1-13.
7. Aulehla-Scholz C, Basaran S, Agaoglu L, Arcasoy A, Holzgreve W, Miny P, Ridolfi F, Horst J. Molecular basis of beta-thalassemia in Turkey: Detection of rare mutations by direct sequencing. Hum Genet 1990;84:195-7.

8. Başak AN, Özçelik H, Özer A, Tolun A, Aksoy M, Ağaoğlu L, Ridolfi F, Ulukutlu L, Akar N, Gurgey A, Kırdar B. The molecular basis of beta-thalassemia in Turkey. Hum Genet 1992;89:315-8.
9. Atalay EÖ, Çırakoğlu B, Dinçol G, Atalay A, Kılınç Y, Aytekin H, Yüregir GT, Arpacı A, Bermek E, Aksoy M. Regional distributions of β-thalassemia mutations in Turkey. Int J Hematol 1993;57:207-11.
10. Tadmouri GO, Garguier N, Demont J, Perrin P, Basak AN. History and origin of beta-thalassemia in Turkey: sequence haplotype diversity of beta-globin genes. Hum Biol 2001;73:661-74.
11. Tadmouri GO, Basak AN. Beta-thalassemia in Turkey: a review of the clinical, epidemiological, molecular, and evolutionary aspects. Hemoglobin 2001; 25:227-39.
12. Atalay E, Çırakoğlu B, Erdoğan G, Bermek E, Aksoy M. Prenatal diagnosis of a beta-globin gene IVS 1nt/110 mutation in chorionic villus from a mosaic triple-X mother with high HbA2. Prenat Diagn 1990;10:343-4.
13. Tüzmen S, Tadmouri GO, Özer A, Baig SM, Özçelik H, Başaran S, Başak AN. Prenatal diagnosis of beta-thalassaemia and sickle cell anaemia in Turkey. Prenat Diagn 1996;16:252-8.
14. Gürgey A, Beksaç S, Gümrük E, Çakar N, Mesci L, Altay S, Öner C, Altay Ç. Prenatal diagnosis of hemoglobinopathies in Turkey: Hacettepe experience. Pediatr Hematol Oncol 1996;13:163-6.
15. Çürük MA, Kılınç Y, Evruke C, Özgünen FT, Aksoy K, Yüregir GT. Prenatal diagnosis of HbH disease caused by a homozygosity for the alpha2 polyA (AATAAA--->AATAAG). Hemoglobin 2001;25: 255-9.
16. Keskin A, Turk T, Polat A, Koyuncu H, Saracoglu B. Premarital screening of beta-thalassemia trait in the province of Denizli, Turkey. Acta Hematol 2000; 104:31-3.
17. Bolaman Z, Enli Y, Köseoğlu M, Koyuncu H, Aslan D. Prevalance of beta thalassemia trait in Denizli. Turk J Hematology 2001;18:85-8.
18. Sözmen M, Uysal Z, Yeşil N, Akar N, Arcasoy A. Denizli'de anormal hemoglobin ve hemoglobin A2 yüksekliği ile karakterize beta talasemi taşıyıcılığı araştırması. Ankara Tıp Mecmuası 1990;43:959-64.
19. Poncz M, Solowiejczky D, Harpel B, Mory Y, Schwartz E, Surrey S. Construction of human gene libraries from small amount of peripheral blood. Hemoglobin 1982;6:27-36.
20. Old JM. Hemoglobinopathies: Community clues to mutation detection. In: Elles R (ed). Molecular Diagnosis of Genetic Diseases. New Jersey: Humana Press Inc, 1996:169-83.
21. Gülesken S, Ören H, Vergin C, Sanlı N, Gulen H, Ucar C, İrken G. Mutational analysis of beta-thalassemia cases from the Aegean region of Turkey using an allele-specific oligonucleotide hybridization technique. Acta Hematol 2000;104:181-4.

Address for Correspondence:

Erol Ömer ATALAY, MD

Department of Biophysics
Pamukkale University School of Medicine
Denizli, TURKEY

e-mail: *eatalay@pamukkale.edu.tr*