Research Article

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The Hematological and Molecular Spectrum of α-Thalassemias in Turkey: The Hacettepe Experience

Türkiye'de Alfa Talasemilerin Hematolojik ve Moleküler Spektrumu: Hacettepe Deneyimi

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Abstract:

Objective: The spectrum of α-thalassemias correlates well with the number of affected α-globin genes. Additionally, combinations of the several non-deletional types of mutations with a large trans deletion comprising the 2 α-globin genes have an impact on the clinical severity. The objective of this study was to analyze the hematological and molecular data of 35 patients with Hb H disease from a single center in order to identify the genotypes of Hb H disease and genotype-phenotype correlations.

Materials and Methods: Herein, we report the hematological and mutational spectrum of patients with Hb H disease (n=35). Additionally, genotypes of α-gene mutations of 78 individuals, who were referred to our institution for α-gene screening, were analyzed.

Results: Supporting the previous data from Turkey, $-\alpha^{3.7}$ was the most common mutation among patients with Hb H disease (62.8%) and in the other 78 subjects (39.7%). Of the patients with Hb H disease, the most common genotypes were $-\alpha^{3.7}/--^{20.5}$, $-\alpha^{3.7}/--^{26.5}$, and $-\alpha^{3.7}/--^{17.5}$ in 10 (28.6%), 6 (17.1%), and 6 (17.1%) patients, respectively. Another small deletion, -4.2 alpha, and several non-deletional types of α-gene mutations, namely α (-5nt): IVS-I donor site (GAG.GTG.AGG->GAG.G----); α (PA-2): AATAAA>AATGGA, and α (cd59): GGC->GAC, were found to be associated with Hb H disease when present at trans loci of one of the large deletions given above. The combinations consisting of 1 non-deletional and 1 of the large deletional types of mutations ($\alpha^T\alpha/--$) at trans loci were found to result in a more severe phenotype compared to the genotypes composed of 1 small trans deletion of a large deletion ($-\alpha/--$). The combination of α (Cd59) and – in trans was associated with severe phenotype and the disease was associated with an increase in Hb Bart's level with null Hb H. In spite of the presence of 2 intact α-globin genes, homozygosity for PA-2 mutation resulted in severe Hb H disease.

Conclusion: This study indicated that Hb H disease is not rare in Turkey and its genotype is quite heterogeneous.

Key Words: Molecular, Mutation, α-Thalassemia, Turkey

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Özet:

Amaç: Alfa (α) talasemilerin farklı klinik spektrumundan etkilenen α-globin gen sayısı sorumludur. Ayrıca delesyonel olmayan mutasyonların, iki α-globin geninin birden etkilendiği büyük delesyonel mutasyonlarla kombinasyon oluşturmasının da hastalığın klinik şiddetinde etkisi bulunmaktadır.

Gereç ve Yöntemler: Burada Hb H hastalarımızın (n=35) hematolojik ve mutasyonel spektrumunu sunmaktayız. Buna ek olarak, merkezimize α-globin geninde mutasyon varlığı taraması için merkezimize gönderilen ve α-globin geni mutasyonu taşıyan 78 bireyin bulguları analiz edilmiştir.

Bulgular: Çalışmamızda daha önce bildirilenleri destekler şekilde Hb H hastası grubunda (%62,8) ve 78 bireyde (%39,7) en sık mutasyon -$\alpha^{3,7}$ olarak bulunmuştur. Hemoglobin H hastalarımızda en sık genotipler -$\alpha^{3.7}$/--$^{20.5}$; -$\alpha^{3,7}$/--^{26,5} ve -$\alpha^{3,7}$/--^{17,5} olarak sırasıyla 10 (%28,6), 6 (%17,1) ve 6 (%17,1) sıklıklarda bulunmuştur. Diğer bir küçük delesyon olan -4.2 (Asya tipi), delesyonel olmayan α-globin mutasyonları α (-5nt): IVS-I donor site (GAG.GTG.AGG->GAG.G-----); α (PA-2): AATAAA>AATGGA ve α (cd59): GGC->GAC; translarında büyük delesyonel bir mutasyon bulunduğunda Hb H hastalığına neden olduğu görülmüştür. Delesyonel olmayan mutasyonla büyük delesyonel tipte mutasyonların kombinasyonlarının ($\alpha^T\alpha$/--), sadece delesyonel mutasyonların kombinasyonları sonucu gelişen Hb H hastalarına göre kliniklerinin daha şiddetli olduğu gözlenmiştir (-α/--). α(Cd59) ve -- trans birlikteliğinde, (α (Cd59)/--), daha ağır bir fenotip izlenmiştir ve bu durumda Hb H bulunmayıp, hastada Hb Bart's yüksek olarak ölçülmüştür. Homozigot PA-2 mutasyonu olan hastalar (α PA-2/α PA-2) ağır fenotipte Hb H hastaları olarak gözlenmiştir.

Sonuç: Çalışmamız Hb H hastalığının ülkemizde nadir olmadığına ve genotipinin heterojen olduğuna işaret etmektedir.

Anahtar Sözcükler: Moleküler, Mutasyon, Alfa talasemiler, Türkiye

Introduction

α-Thalassemia results from a genetic defect in α-globin chain synthesis, often as a consequence of deletional mutations and less frequently due to non-deletional types of mutations [1,2]. α-Thalassemias may occur worldwide; however, they are seen more commonly among populations in South East Asia, the Mediterranean region, and the Middle East [1]. The α-globin gene is located on the short arm of chromosome 16 (16p13.3) and normally there are 4 α-globin gene copies in an individual, with 2 in each allele [3]. The phenotype of α-thalassemias is directly related to the number of α-globin genes affected. α^+-Thalassemias designate the status of deletion in one of the paired α-globin genes (-α/αα), whereas in α^0-thalassemias both of the paired α-globin genes are deleted (--/αα). Heterozygous α^+-thalassemia usually causes a silent carrier state. On the other hand, heterozygous α^0-thalassemia (--/α) and homozygous α^+-thalassemia (-α/-α) result in hematological findings similar to α-thalassemia trait, except for the Hb A_2 value, which is at the normal level or below the normal level in α-thalassemia. The co-existence of both α^+-thalassemia and α^0-thalassemia (-α/--) results in hemoglobin H (Hb H) disease [1]. There are also non-deletional types of mutations ($\alpha^T\alpha$) resulting in Hb H disease, when a large deletional type of mutation (--) co-exists in trans ($\alpha^T\alpha$/--) [4,5].

The most common deletional mutations causing α^+-thalassemia are -$\alpha^{3.7}$ and -$\alpha^{4.2}$, whereas the common deletional mutations causing α^0-thalassemias are of 20.5-kb deletion, approximately 17.5-kb deletion (-MED-I), greater than 26.5-kb deletion (-MED-II), and approximately 18-kb deletion (-SEA) [1,4,6]. MED-II has previously been reported in a few Turkish families and from other Mediterranean populations [4].

In this study, the hematological and molecular data of 35 patients with Hb H disease from a single center were analyzed and reported in order to identify the genotypes of Hb H disease and genotype-phenotype correlations, and also to create awareness that Hb H disease is not a rare entity in Turkey.

Materials and Methods

Of the 788 patients who were diagnosed with thalassemia between 1981 and 2014 at our institution, 138 (17.5%) were diagnosed with Hb H disease (Table 1). Unfortunately, from those 138 patients only a total of 35 had genotype data available; those 35 were included in the current study. Splenomegaly was detected at diagnosis, during physical examination, or by ultrasonography in 40% of the patients with Hb H disease. The transfusion histories of patients with Hb H were recorded from patients' files. Of the patients with Hb H disease, 18% received erythrocyte transfusion at least once, and 82% had no transfusion history at diagnosis and received no transfusion during follow-up. The number of transfusions ranged between

1 and 24. One patient was on a chronic transfusion program, whereas the other patients were transfused occasionally. Ethical committee approved this study.

Excluding the patients with Hb H disease, of the individuals screened for α-thalassemia mutations, 78 were found to carry an α-thalassemia mutation. The indications of α-thalassemia mutational screening among those 78 individuals were either having hypochromic microcytic erythrocytes, with normal iron status and Hb A_2 below 3.5%, or being the available parent of a patient with Hb H disease.

Results of hematological studies and red cell indices were analyzed. For discussion purposes, values prior to splenectomy or erythrocyte transfusion were taken into consideration. Hemoglobin A_2, Hb F, and Hb H values were measured with the previously described methods [7] or high-performance liquid chromatography with the Bio-Rad Variant II system. Supravital stains for Hb H inclusions were examined in all cases [8].

Prior to 2008, α-thalassemia mutations were identified with previously described methods [7,8,9,10,11,12,13]. After 2008, mutation analyses for the α-globin gene were evaluated with the α-Globin Strip-Assay (ViennaLab, Austria), based on the reverse-hybridization technique used for detection of the 21 most common α-thalassemia mutations in the Mediterranean region. Of the 35 patients with Hb H disease, 25 have been reported previously [7].

The obtained data were evaluated with SPSS 21 (IBM Corp., Armonk, NY, USA). Normality test was performed to determine if the data were distributed in a normal fashion. For comparison between groups of more than 2, one-way ANOVA test was used. Statistical significance was determined as p values <0.05.

Results

Of the 35 patients with Hb H disease, the age range was 1.5-50 years at diagnosis (mean: 15.9±12.9 years). The mean values of red blood cell indices at diagnosis are summarized in Table 2a. A total of 10 different genotypes were detected in 35 patients with Hb H disease (Tables 2b and 2c.).

Of the 35 patients with Hb H disease, 22 (62.8%) and 18 (51.4%) were found to have $-\alpha^{3.7}$ or $--^{20.5}$ alleles, respectively (Table 3). The most common genotype was $-\alpha^{3.7}/--^{20.5}$ in 10 (28.6%) of the patients, followed by $-\alpha^{3.7}/--^{26.5}$ in 6 (17.1%) and $-\alpha^{3.7}/--^{17.5}$ in 6 (17.1%). The most common 3 genotypes were distributed among 22 of the 35 patients, representing 62.8% of all genotypes found in patients with Hb H disease. The numbers of Hb H patients having other genotypes were too small to make any statistical analysis; therefore, comparison of the hematological data was made only among the patients with the 3 most common above-mentioned genotypes.

Statistical analyses of the mean values of red cell indices showed no significant difference among these 3 common genotypes. Hemoglobin F level was found significantly higher in $-\alpha^{3.7}/--^{17.5}$ patients (p=0.041), whereas Hb H levels were significantly lower among patients with this genotype compared to the $-\alpha^{3.7}/--^{20.5}$ and $-\alpha^{3.7}/--^{26.5}$ genotypes (p=0.036). Hemoglobin A_2 levels were similar among these 3 genotypes.

Of the patients with Hb H disease, 26 (74.3%) were found to have deletional types of mutations, whereas 9 (25.7%) were found to have non-deletional types of mutations. Comparison of the hematological data of the Hb H patients showed that the group of patients with a genotype consisting of non-deletional types of mutations with a large trans deletion ($\alpha\alpha^T/--$) had statistically lower hemoglobin values (p=0.007) compared to those who had deletional types of mutations with a large trans deletion ($-\alpha/--$) (Table 4). On the other hand, the mean of Hb H levels was significantly higher in the former patients (18.1± 8.3 vs. 7.4±4.7; p=0) than the latter (Table 4). In the examination of the 78 individuals with α-thalassemia mutations other than Hb H disease, the most common genotype was $-\alpha^{3.7}/\alpha\alpha$ in 31 patients (39.7%) (Table 5). The most common non-deletional genotype was $\alpha(PA\text{-}1)/\alpha\alpha$ in 5 of the individuals (6.4%). Of the 78 subjects, 34 (43.5%) and 21 (26.9%) were found to have $-\alpha^{3.7}$ or $--^{20.5}$ alleles, respectively (Table 5).

Discussion

The incidence of deletional α-thalassemia ($-\alpha/\alpha\alpha$) among newborns screened by globin gene mapping from samples obtained from cord blood at birth has been reported to be 3.6% in Turkey [14]. In other reports, the chromatographic analyses of cord blood samples of newborns in Turkey suggested that $-\alpha/\alpha\alpha$ or ($\alpha^T\alpha$) thalassemia incidence was between 2.9% and 4.1% [15,16].

In a recent report from Antakya-Hatay, a city in the southern part of Turkey, 300 individuals with moderate anemia, microcytosis, and normal iron levels were tested for α-thalassemia by the aid of α-globin strip assay; of these, 97 were found to have at least 1 mutation in 4 of the α-globin genes [17]. Of these patients, the most common mutation was $-\alpha^{3.7}$ (57.3%) [17]. Similarly, Öner et al. and Çürük reported $-\alpha^{3.7}$ as the most common α-thalassemia gene

Table 1. The distribution of β- and α-thalassemias between 1981 and 2014 in the Hacettepe University Division of Pediatric Hematology.

Disease	n (%)
β-thalassemia major/intermedia	650 (82.5)
Hb H	138 (17.5)
Total	788 (100)

Table 2a. The age and hematological data of patients with Hb H disease with molecular diagnosis.

Patients with Hb H		Age at diagnosis (years)	Hb (g/dL)	MCV (fL)	MCH (pg)	MCHC (g/dL)	RBC (x10^{12}/L)	RDW	Hb A_2 (%)	Hb F (%)	Hb H (%)
Total	n=35										
	Mean ± SD	15.9±12.9	9.3±1.6	63.1±9.7	17.7±1.8	30.9±2.3	4.7±0.8	22.5±8.5	1.2±0.4	1.3±0.9	10.3±7.5
	Range	1.5-50	6.7-13.7	48-98	15.3-20.9	28.2-35.8	2.8-6.4	9.5-34.9	0.5-2	0-4.3	1.4-34

Table 2b. The age and hematological data of patients with Hb H disease with the 3 most common genotypes.

Genotype		Age at diagnosis (years)	Hb (g/dL)	MCV (fL)	MCH (pg)	MCHC (g/dL)	RBC (x10^{12}/L)	RDW	Hb A_2 (%)	Hb F (%)	Hb H (%)
-$\alpha^{3.7}$/--$^{20.5}$	n=10										
	Mean ± SD	14.8±9.6	9.8±1.6	64.6±9.6	16.6±1.2	29.5±1.1	4.9±0.4	23.9±2.4	1.4±0.4	0.9±0.6	9.9±5.2
	Range	1.5-30	8.1-12.3	51.4-77	15.3-17.5	28.2-30.3	4-5.3	21.2-25.7	0.9-2	0.5-2.2	2.9-17
-$\alpha^{3.7}$/--$^{26.5}$	n=6										
	Mean ± SD	18±6.5	9.9±1.5	61.5±7.3	17.5±1.7	30.5±1.5	5.4±1.1	21.8±11.7	1±0.2	0.7±0.4	8±4.8
	Range	8-28	7.5-11.6	52-72	15.8-19.2	29.1-32.1	3.9-6.4	13-32.8	0.9-1.2	0-1.1	1.5-15.4
-$\alpha^{3.7}$/--$^{17.5}$	n=6										
	Mean ± SD	13.6±15.3	9.5±0.3	56.3±5.3	19.2	35.8	4.9±0.5	11.5	1.5±0.3	2.4±1.3	3.3±1.7
	Range	2-43	9-9.9	48-63	19.2	35.8	4.2-5.8	11.5	1.2-1.9	0.6-4.3	1.4-6
p		>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	0.041	0.036

Table 2c. The age and hematological data of patients with Hb H disease associated with rare mutations.

Genotype		Age at diagnosis (years)	Hb (g/dL)	MCV (fL)	MCH (pg)	MCHC (g/dL)	RBC (x10^{12}/L)	RDW	Hb A_2 (%)	Hb F (%)	Hb H (%)
α (-5nt*)/--$^{20.5}$	n=3										
	Mean ± SD	13±2.6	8.4±0.6	68.3±2.5	NA	NA	4.6±0.8	NA	0.8±0.2	1.5±0.9	23.2±9.6
	Range	10-15	7.9-9	66-71			4-5.6		0.6-0.9	1-2.5	15.5-34
-α$^{4.2}$/--$^{20.5}$	n=2										
	Mean ± SD	27±29.6	9.7±2.3	64.8±5.4	20.9	30.4	4.4±1.4	19	0.9±0.5	0.8±0.3	6.5±3
	Range	6-48	8.1-11.4	61-68.6	20.9	30.4	3.4	19	0.6-1.3	0.6-1	4.4-8.6
α (PA-2**)/--$^{20.5}$	n=2										
	Mean ± SD	2.3±0.4	7.8±0.4	53±2.8	NA	NA	4.3±0.1	NA	0.7±0.1	1.1±0.6	13.8±2.5
	Range	2-2.5	7.5-8.1	51-55			4.2-4.3		0.6-0.7	0.7-1.5	12-15.5
-α$^{4.2}$/--$^{17.5}$	n=2										
	Mean ± SD	38.5±16.3	9.1±1.3	65.5±2.1	NA	NA	4.2±0.6	NA	1.5±0.5	1.2±0.9	10.7±3.8
	Range	27-50	8.2-10	64-67			3.7-4.6		1.1-1.8	0.5-1.8	8-13.4
α (PA-2**)/α (PA-2)	n=2										
	Mean ± SD	8±2.8	6.8±0.2	68±0	NA	NA	3.6±0.6	NA	0.8±0.1	1.2±0.4	14.1±8
	Range	6-10	6.7-6.9	68-68			3.2-4.1		0.7-0.9	1-1.5	8.4-19.7
α (cd59 ***) /--$^{20.5}$	n=1										
	Value	8	13,700	98	NA	NA	2.8	NA	0.8	NA	10.5****
α (-5nt*)/--$^{17.5}$	n=1										
	Value	12	8.2	64	NA	NA	4.8	NA	0.5	2.9	28

*: α (-5nt): IVS-I donor site (GAG.GTG.AGG->GAG.G-----); **: α (PA-2): AATAAA>AATGGA; ***: α (cd59): GGC->GAC; ****: This value indicates Hb Bart's but not Hb H for this particular patient.

that was associated with Hb H disease in 25 and 32 patients, respectively [7,18]. Our study is compatible with the above stated previously published data pointing out that $-\alpha^{3.7}$ has been the most common genotype among patients with Hb H disease (62.8%).

In our study, among Hb H patients, the second most common allele was $--^{20.5}$ (51.4%). This finding is in accordance with the other reports from Turkey [7,18,19]. A hydrops fetalis case due to α-thalassemia associated with homozygosity of $--^{20.5}$ was also previously reported from Turkey [20].

In the current study, the $-^{MED-II}$ deletion ($--^{26.5}$) was found as the third most common allele among patients with Hb H disease (25.7%), which was followed by $--^{MED-I}$ deletion ($--^{17.5}$) at 17%. Contrary to our observation, the $--^{MED-I}$ mutation ($--^{17.5}$) has been reported as the second most common type of allele by Guvenc et al. with 15.11% frequency among the population of Adana, a city in the southern part of Turkey [21]. This is probably related to the homogeneity of the population studied in that publication.

The $--^{MED-II}$ deletion has been known as a genotype more common among Turkish populations [4], and it was found as the third most common allele in our study.

All of these studies suggest that the molecular pathology of Hb H disease is heterogeneous and, according to our study, the most common genotypes associated with Hb H in 35 patients who were referred to us from all over Turkey are as follows: $-\alpha^{3.7}/--^{20.5}$ (28.6%), $-\alpha^{3.7}/--^{26.5}$ (17.1%), and $-\alpha^{3.7}/--^{17.5}$ (17.1%) (Table 2b).

In the current study, 25.7% of the patients with Hb H disease who had a combination of large deletional and non-deletional ($\alpha\alpha^{T}/--$) mutations were found to have statistically significantly lower Hb and higher Hb H levels compared to those of patients having combinations of large and small deletional ($-\alpha/--$) types of mutations (Table 4). This finding was compatible with the previously published data [1,2,3]. This study revealed the presence of 3 different non-deletional types of mutations, namely the (-5nt), PA2, and C59 mutations. It seemed that the most common non-deletional type of combination involved in Hb H was (-5nt/--), which was found in 3 patients (8.6%) in the current study. Contrary to this, α (PA-2)/$--^{MED-II}$ was the most frequent non-deletional combination in a regional study by Çürük [18]. It was interesting that in spite of the presence of 2 intact α-globin genes, homozygosity for PA-2 mutation (α PA-2/α PA-2) resulted in severe Hb H disease in 2 patients (Table 2c); this was discussed elsewhere [7].

Table 3. Distribution of mutations in 35 patients with Hb H (70 chromosomes).

Genotype	Number of chromosomes affected
$-\alpha^{3.7}$	22
$-\alpha^{4.2}$	4
α (PA-2)	6
α (-5nt)	4
α (cd59)	1
$--^{20.5}$	18
$--^{17.5}$	9
$--^{26.5}$	6
Total	70

Table 4. The comparison between hematological parameters of patients with Hb H disease with deletional and non-deletional types of mutations.

Genotype	Hb (g/dL)	RBC (x10^{12}/L)	MCV (fL)	Hb A_2 (%)	Hb F (%)	Hb H (%)
Combination of deletional mutations* (n=26)	9.7±1.3	4.9±0.7	61.9±7.8	1.3±0.4	1.2±0.9	7.4±4.7
Combination of deletional and non-deletional mutations** (n=9)	8.4±2	4.1±0.8	67.6±13.1	0.7±0.1	1.5±0.7	18.1±8.3
p	0.007	0.026	>0.05	0	>0.05	0.001

*Of these 26 patients, 8 were below 10 years of age.
**Of these 9 patients, 4 were below 10 years of age.

Table 5. The distribution of deletional and non-deletional types of α-thalassemia mutations in 78 individuals.

Genotype	n (%)
$-\alpha^{3.7}/\alpha$	31 (39.7)
$--^{20.5}/\alpha\alpha$	21 (26.9)
$--^{26.5}/\alpha\alpha$	8 (10.3)
α (PA-1)/αα	5 (6.4)
α (Cd59 G>A)/αα	4 (5.1)
α (IVS 1-5 nt)/αα	3 (3.8)
$-\alpha^{3.7}/-\alpha^{3.7}$	2 (2.6)
α (PA-2)/αα	1 (1.3)
α (Cd14 G>A)/αα	1 (1.3)
α (Cd14 G>A)/$-\alpha^{3.7}$	1 (1.3)
$-\alpha^{17.5}/\alpha\alpha$	1 (1.3)
Total	78 (100)

In this study, we did not find any of the previously described α-gene mutations from Turkey, such as -THAI, --FIL, init.cd, Cd 19, Hb Icaria, Hb Pakse, or Hb Koya Dora [14,16,17,18,19,21]. In a previous study from our center, the rate of unidentifiable mutations among individuals with α-thalassemia mutations was reported to be 2.72% [22]. In this study, all of the mutations among patients with Hb H disease were known mutations. In the previous study from our center, among individuals with α-thalassemia major, the most common 3 mutations were distributed among 69.39% of the patients [22]. In this study, it was shown that the most common 3 genotypes associated with Hb H accounted for almost 63% of the study group.

In the previous reports by Altay and by Akar and Altay, related to National Hemoglobinopathy Registry data, Hb H was reported to be 3.6% (n=103) of all hemoglobinopathies in Turkey [22,23]. In our cohort study from a single center, it was shown that Hb H disease was diagnosed in 17.5% of the total 650 thalassemic patients (Table 1). The latest figure for α-thalassemia major in Turkey was reported to be 57% of 5500 patients with hemoglobinopathies [24,25]. Therefore, according to the data of our center as stated above, the total number of Hb H patients in Turkey should be around 550. The discrepancy in the rates of Hb H between 2002 data and the current study may derive from the higher awareness of the disorder in some centers in recent years, more accurate diagnoses, and/or developments in the diagnostic tools of Hb H disease and/or an increase in referral rates of anemic patients from peripheral to tertiary centers like ours. Therefore, if the figure of the current study reflects a more accurate value of the number of Hb H cases, we may expect to diagnose more patients in the near future.

In conclusion, as our center is a referral center in the mid-Anatolia region with a patient profile from all over the Turkey, the results of our study may represent the Hb H disease rates among the overall Turkish population. Some of the data of this study were in agreement with previous reports [7,8,9,16,17,18,19,20], and our current study also indicated that the molecular spectrum of α-thalassemias is quite heterogeneous in Turkey, as all together 9 deletional and non-deletional mutations and 10 combinations of them were found to be associated with Hb H disease. In previous reports, the mutational spectra were reported to be less heterogeneous among smaller populations, such as among Cypriots and Iraqi Turks [26,27]. Although in this study the molecular pathology of Hb H disease has been addressed, the frequencies of rare genotypes associated with α-thalassemia requires more patients and further population studies, since most of the individuals screened for that purpose in the current study were parents of the patients with Hb H disease, a limiting factor in prediction of the population frequencies of several genotypes. This study also showed that Hb H disease is not uncommon in Turkey; therefore, this disease should be kept in mind in discussion of microcytic anemias and all efforts should be made for correct diagnosis of α-thalassemias. Detection of new cases will be helpful in determining the allele frequencies of different α-thalassemia mutations.

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Conflict of Interest Statement

The authors of this paper have no conflicts of interest, including specific financial interests, relationships, and/or affiliations relevant to the subject matter or materials included.

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