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Molecular Testing for Thalassemia: Mutation Detection According to Referral Reasons and Demographic Data

Şule Altıner[1], Halil Gürhan Karabulut[1], Sadiye Ekinci[1], Arzu Vicdan[1], Nüket Kutlay[1], Timur Tuncalı[1], Ajlan Tükün[1,2], Hatice Ilgın Ruhi[1]

ABSTRACT

Objective: Thalassemia is the most common inherited blood disorder worldwide and an important public health problem in Mediterranean countries such as Turkey. In this study, we aimed to define mutation detection rates according to referral reasons based on molecular testing results.

Materials and Methods: The data of 315 patients tested for thalassemia using the reverse dot-blot method between 2007 and 2017 at the Department of Medical Genetics, Ankara University School of Medicine, were analyzed.

Results: The most frequent mutations were 3.7-kb deletion and IVS1.110 [G>A] for alpha (α)- and beta (β)-thalassemia, respectively. Statistical correlation was found between the samples identified after clinical indication and the mutation detection rate in α-thalassemia (Pearson chi-square two-sided p=0.006). Moroever, a statistically significant correlation was detected between the β-thalassemia mutation rate and positive family history (continuity-to-correction two-sided p=0.002).

Conclusion: Our results highlight the importance of positive family history and evaluation of hematologic parameters and consanguinity in mutation detection for thalassemias.

Keywords: Thalassemia, Turkey, referral reason, mutation

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[1]Department of Medical Genetics, Ankara University Faculty of Medicine, Ankara, Turkey
[2]Department of Medical Genetics, Düzen Laboratories Group, Ankara, Turkey

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Correspondence
Şule Altıner,
Ankara University Faculty of Medicine, Department of Medical Genetics, Ankara, Turkey
Phone: +90 312 595 81 51
e-mail: bicers@ankara.edu.tr

INTRODUCTION

Thalassemias are inherited in an autosomal recessive pattern caused by decreased or abolished production of one of the two globin chains of the hemoglobin molecule, alpha (α)-globin chain (HBA) or beta (β)-globin chain (HBB), during prenatal and postnatal life. As a result of the decreased expression of the affected globin chain, the amount of the unaffected globin chain relatively increases. The imbalanced ratio of the α and β chains is the underlying pathophysiology that causes characteristic abnormalities in laboratory tests (1). The β-globin gene (HBB) is located on 11p15.4. β-Thalassemias are caused by point mutations at an incidence rate of 95% (2). On the other hand, the α-globin gene cluster has two copies of α genes (HBA1 and HBA2) on each chromosome 16 (16p13.3), and 95% of α-thalassemia cases are caused by deletion (2).

Thalassemia is the most common inherited blood disorder worldwide. According to World Health Organization reports, approximately 60,000 infants are born with thalassemia major every year. β-Thalassemia is commonly found in the Mediterranean, central Asia, India, Africa, South America, and the Middle Eastern countries (3). Moreover, α-thalassemia is common in southern and southeastern Asia and southern China (4).

In this study, we aimed to determine the referral reason with the highest possibility of mutation detection in patients with thalassemia.

MATERIALS and METHODS

In this study, the data of 315 patients tested for thalassemia mutations between 2007 and 2017 at the Department of Medical Genetics, Ankara University School of Medicine, were analyzed according to referral reasons, test results, and demographic data. This was a retrospective descriptive study, and ethics committee approval for the study was obtained from the Ankara University Medical Faculty Ethics Board on August 6, 2020 (No. İ6-379-20).

A total of 367 mutation tests were performed using the reverse dot-blot method. We performed α only, β only, and both α and β mutation analyses in 194, 69, and 52 cases, respectively.

Iron deficiency was excluded for all the patients with microcytic anemia before testing. The patients were divided into two groups according to the type of test performed, namely the α (n=246) and β (n=121) mutation tests. Then, each group was divided into two subgroups according to referral reason as follows: 1) samples identified

after clinical indication and 2) samples identified through a prevention program (or premarital screening).

Samples identified after clinical indication were included in etiologal investigations to assess for changes in hematologic parameters and positive family history. Patients with suspected mutation carrier status in the premarital screening test and couples with thalassemia carrier history in either of the partners were grouped as samples identified through the prevention program.

Hematologic parameters were indicated as the presence or absence of changes in blood count parameters in the patients analyzed for α only mutations. Blood count parameters followed by hemoglobin (Hb) electrophoresis/high-performance liquid chromatography were used for β mutations (lower limit of hemoglobin: 12.5 g/dL for women and 13.5 g/dL for men, red blood cell count: 4.5×1012/L, MCV: 70 fL, MCH: 27 pg/cell, and upper limit of HbA2: 3%).

SPSS 20 software was used for the data analysis. Chi-square statistics was used to test the association between the two variables. A p value <0.05 was considered statistically significant.

RESULTS

A total of 315 patients (male, n=126 and female, n=189) were enrolled in the study. The mean ages of the patients in the α- and β-thalassemia mutation analysis groups were 30.52±13.3 years (median: 29 years, range: 2–67 years) and 27.07±14 years (median: 27 years, range: 1–66 years), respectively.

Parental consanguinity was observed in 59 patients (23.9%) who were tested for α-thalassemia mutation. At least one mutation was detected in 18 patients, and homozygous mutations were harbored by five patients. On the other hand, of the 33 patients (27.2%) who had consanguineous parents, 14 tested positive for β-thalassemia mutation, of whom seven had homozygous mutations.

α-Thalassemia mutations: We evaluated 246 patients for α-thalassemia mutations and found 11 to have hemoglobin H disease, while 28 had thalassemia trait and 38 were silent carriers. In addition to these patients, three patients had α-globin gene triplication (Table 1). One of them solely had an α-globin gene triplication, whereas Mediterranean (MED) deletion accompanied the triplication in the second patient. The third patient was evaluated for both α- and β-thalassemia mutations and found to have mutations in both globin genes (α-globin gene triplication/αα, IVS1.110 [G>A]/β).

β-Thalassemia mutations: We evaluated 121 patients for β-thalassemia mutations. Nine patients had homozygous or compound heterozygous mutations, 42 had heterozygous mutations, and one had a homozygous deletion (Table 2).

Both α- and β-thalassemia mutations: We evaluated 52 patients for both α- and β-thalassemia mutations. Only one was found to have both mutations (α-globin gene triplication/αα, IVS1.110 [G>A]/β).

Mutant alleles: For α genes, presence of a mutation in one or both α genes (α1, α2) on the same chromosome is accepted as a mutant allele. Therefore, mutations in the α1 and α2 genes are not individually evaluated. The number of mutant alleles was 107 for α-thalassemia and 62 for β-thalassemia. The most frequent mutant alleles carried 3.7-kb deletion (n=58, 54.2%), MED deletion (n=15,

Table 1. α-Thalassemia mutation analysis results

Result of α thalassemia mutation analysis	Patient	
	n	%
-α(3.7)/--(MED)	5	2.032
-α(3.7)/--(20.5)	4	1.626
--(20.5)/-α(a2 cd 19[-G])	1	0.406
-α(a2IVS1-5nt)/--(MED)	1	0.406
--(MED)/-α(anti-3.7)	1	0.406
-α(3.7)/-α(3.7)	12	4.878
--(MED)/αα	8	3.252
--(20.5)/αα	5	2.032
-α(3.7)/-α(4.2)	1	0.406
-α(a2IVS1-5nt)/-α(3.7)	1	0.406
-α(3.7)/-α(a2 poly A-2 [AATAAA>AATGAA])	1	0.406
-α(3.7)/αα	22	8.943
-α(a2 IVS 1-5nt)/αα	9	3.658
-α(a1 cd 59 [G>A][Hb Adana])/αα	4	1.626
-α(4.2)/αα	1	0.406
-α(a1 cd14 [G>A])/αα	1	0.406
-α(a2 poly A-2 [AATAAA>AATGAA]/αα	1	0.406
-α(anti-3.7)/αα	2	0.826
αα/αα	166	67.479

Table 2. β-Thalassemia mutation analysis results

Results of β thalassemia mutation analysis	Patient	
	n	%
IVS 1.110 [G>A]/ IVS 1.110 [G>A]	2	1.652
IVS 2.1 [G>A]/IVS 2.1 [G>A]	2	1.652
HbS/IVS 1.5 [G>C]	2	1.652
IVS 1.6 [T>C]/ IVS 1.6 [T>C]	1	0.826
codon 8 [-AA]/codon 8 [-AA]	1	0.826
HbS/HbS	1	0.826
IVS 1.110 [G>A]/β	18	14.876
codon 8 [-AA]/β	5	4.132
IVS 1.1 [G>A]/β	4	3.305
IVS 1.5 [G>C]/β	3	2.479
IVS 2.1 [G>A]/β	2	1.652
codon 39 [C>T]/β	2	1.652
-30 [T>A]/β	2	1.652
HbS/β	2	1.652
IVS 2.745 [C>G]/β	1	0.826
codon 6 [-A]/β	1	0.826
codon 44 [-C]/β	1	0.826
IVS1.6[T>C]/β	1	0.826
homozygous entire gene deletion	1	0.826
β/β	69	57.024

14%), and a2IVS1-5nt mutation (n=11, 10.2%) for α-thalassemia. On the other hand, IVS1.110 [G>A] (n=22, 35.4%), codon 8 [–AA] (n=7, 11.2%), IVS2.1 [G>A], and HbS (both: n=6, 9.6%) were the most frequent mutations for β-thalassemia in our study.

The comparison of the referral reasons for mutation testing revealed a statistically significant correlation between the samples identified after clinical indication and the mutation detection rate in α-thalassemia. At least one mutation was detected in 40% of the patients referred with clinical indications, and the mutation detection rate was 20% in the rest of the patients (Pearson chi-square two-sided p=0.006). Similarly, α-thalassemia mutation was detected in 13 (52%) of the 25 patients with positive family history and in 67 (30.3%) of the 221 patients without a positive family history (continuity-to-correction two-sided p=0.049). Fifteen patients had MED mutations, which were found in the samples identified after clinical indication in 13 patients (continuity-to-correction two-sided p=0.059).

The statistical analysis results were similar between the referral reasons and mutation detection rates for β-thalassemia mutation. At least one mutation was detected in 52.3% of the patients evaluated after clinical indication, and the mutation detection rate was 18.2% in the remaining patients (continuity-to-correction two-sided p=0.002). In addition, we observed a statistically significant correlation between the β-thalassemia mutation rate and positive family history. β-Thalassemia mutations were detected in 17 (73.9%) of the 23 patients with a positive family history and in 35 (35.7%) of the 98 patients without a positive family history (continuity-to-correction two-sided p=0.002).

DISCUSSION

In our study, the reverse dot-blot method, a practical and cost-effective strategy, was used to detect common deletions and point mutations. The mutation detection rates with this method were 32.5% (80/246 patients) and 42.9% (52/121 patients) for α- and β-thalassemia mutations in our study, respectively.

The IVS1.110 [G>A] substitution was the most prevalent mutation, with a frequency of 47% in all β-thalassemia alleles in the cohort published by Aydınok and Oymak (5). Three different large deletions (–/αα: MED deletions: –17.4, –26.5, and –20.5 kb) and two small deletions (–α/αα: –3.7 and –4.2 kb) were reported to be frequent in α-thalassemia in Turkey (6). In our study, IVS1.110 [G>A] β and 3.7-kb α deletion mutations were found to be the most frequent mutant thalassemia alleles. The partial incompatibility of the mutation frequencies between this study and previous reports may suggest an admixture or indeterminate origin of cohorts and patients recruited in this study.

α-Globin gene triplication was detected in three patients. Carriers of this mutation are known to not show clinical symptoms or significant laboratory changes (7). The robust gene dosage is related with the clinical condition, which is not an unexpected situation, although the β-chain is relatively decreased. Coexistence of an α-globin gene triplication and β-thalassemia increases the severity of the disease owing to the augmented imbalance between the chains (8). In accordance with the literature, one of the patients had a positive history of α-globin gene triplication and normal hematologic parameters. Another patient with both MED deletion and triplication was referred as a suspected mutation carrier in the premarital screening test. In fact, the α-globin gene copy numbers are balanced in this case. The third patient, who had both α-globin gene triplication and IVS1.110 [G>A]/β mutation, had a medical history of nontransfusion-dependent thalassemia. Coinheritance of the α-globin gene triplication with heterozygous β-thalassemia mutation increases the level of chain imbalance, aggravating the clinical manifestation of thalassemia.

In our study, 92 (29.2%) patients had parental consanguinity. At least one thalassemia mutation was detected in 32 of them. Twelve were homozygous, one was compound heterozygous, and the rest were heterozygous. Sixty percent of the patients who had homozygous mutations had parental consanguinity in their families (Pearson chi-square two-sided p=0.002).

In conclusion, the genotype distribution according to the reason for referrals indicated the importance of positive family history and evaluation of hematologic parameters.

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