ORIGINAL ARTICLE

Eastern Journal of Medicine

East J Med 26(1): 117-122, 2021
DOI: 10.5505/ejm.2021.04796

Investigation of Alpha Globin Gene Mutations by Complementary Methods in Antalya

Ibrahim Keser[1*], Tuğba Karaman Mercan[1], Türker Bilgen[2], Osman Alphan Kupesiz[3], Yunus Arikan[4], Duran Canatan[5]

[1]Department of Medical Biology and Genetics, Faculty of Medicine, Akdeniz University, 07079 Antalya, Turkey
[2]Central Laboratory, NABILTEM, Namık Kemal University, Tekirdağ, Turkey
[3]Department of Pediatric Hematology, Akdeniz University, 07079 Antalya,Turkey
[4]Department of Medical Genetics, Faculty of Medicine, Bozok University, Yozgat, Turkey
[5]Akdeniz Blood Diseases Foundation (AKHAV), 07050, Antalya, Turkey

ABSTRACT

Alpha (α) thalassemia is one of the hemoglobinopaties that is inherited by autosomal recessive mode. It is caused by mutations on alpha-1 and alpha-2 globin genes. Deletional type mutations of globin genes have commonly been seen in alpha thalassemias. While small deletional mutations such as -3.7 cause α+-thalassemia, large deletions such as -26.5 -20.5 cause α^0-thalassemia. The objective of our study was to determine the profile of deletional and non-deletional α-globin gene mutations in the Antalya population, Turkey.

In present study, the presence of α-thalassemia mutations were investigated by RDBH (reverse dot blot hybridization) among 250 patients with microcytic anemia and beta globin normal. Some positive and negative cases were confirmed by MLPA (multiplex ligation dependent probe amplification) and at the latest DNA sequencing.

Eight different mutations were determined in 112 (44.8%) of patients in our study. The $-\alpha\alpha^{3.7}$ deletion was the most common mutation(73.3%). Others common mutations were the $-\alpha^{20.5}$ (13.0%) and $-^{MED}$ (6.5%), --FIL (2.4%), Hb Adana (2.4%). The 97.5 % of total mutations consisted of these five mutations. Three patients with Hb H disease were found related with $-\alpha$ 3.7 /-(α) $-^{20.5}$ genotype. One patient (2.04%) had the ααα anti-3.7 gene triplication. Two rare mutations, α2 codon 64 (G>C) (Hb Fontainebleau) and α2 codon 193 (G>A) (Hb G- Waimanalo), were determined by DNA sequencing firstly in Antalya Province, Turkey.

Our results may be valuable to give accurate premarital genetic counseling and to apply classical prenatal and preimplantation genetic diagnosis by the complementary methods such as RDBH, MLPA and DNA sequencing for the screening of alpha thalassemia carriers.

Key Words: Alpha thalassemia, Alpha globin gene, Mutation, MLPA, RDBH, DNA sequencing

Introduction

α-thalassemia is one of the highest frequent hemoglobinopathies and is characterized by deficient or absent synthesis of four alpha-globin polypeptide chains (1). The localization of α-globin genes is on the 16p13.3 region of human chromosome 16 and there are normally 4 copies of α- globin gene (with two in each allele) in an individual (2). Defects on one or more of the four α-globin genes (αα/αα) cause α-thalassemia phenotypes and the inheritance of these mutations are autosomal recessive. The mutations of alpha globin genes are generally evaluated in the two groups. These are seen as general deletion with (α^+) and (α^0) phenotype and non-deletions with point mutation. If there is the accumulation of gamma globin chain with the decrease of alpha globin chain synthesis, Hb Bart's (γ4) occurs in fetuses. Conversely if there is the intensify of beta globin chain, Hb H (β4) disease occurs in adults (3,4). A mutation in any one of copies of four alpha genes don't cause any clinical effect, but two mutant copies of them cause microcytic and hypochromic anemia. In either case, Hb A2 level is normal. The screening and identification of alpha gene mutations can be solution for prenatal diagnosis and realistic genetic counseling in thalassemic regions at risk.

Turkey is a bridge between Asia, Africa and Europe not only geographically but also human immigrations, espacially from east countries that are the most prevelant of thalassemia carriers to west countries (5). In this regards, Turkey is similar to a "the historical bridge of thalassemia". Many studies were done to

*Corresponding Author: Ibrahim Keser, Department of Medical Biology and Genetics, Faculty of Medicine, Akdeniz University, 07079 Antalya, Turkey
E-mail: ibrahimkeser44@gmail.com, keser@akdeniz.edu.tr, Phone: 0090 (242) 249 69 73, Fax:0090 (242) 249 69 03
ORCID ID: Ibrahim Keser: 0000-0002-5321-0701, **Tuğba Karaman Mercan:** 0000-0003-3341-9513, **Türker Bilgen:** 0000-0002-3015-0929, **Osman Alphan Kupesiz:** 0000-0001-8827-5567, **Yunus Arikan:** 0000-0001-5585-6795, **Duran Canatan:** 0000-0001-8128-8269
Received: 04.09.2020, **Accepted:** 09.12.2020

Table 1. The localization numbers and positions for the 21 known mutations of alpha globin genes on kit

No on Strip	Location	Mutation
1	-α3.7	Gene deletion (Single)
2	-α4.2	Gene deletion (Single)
3	(α) 20.5	Gene deletion (Double)
4	--MED	Gene deletion (Double)
5	α2 IVS1	Deletion (5 bp)
6	ααα anti-3.7	Gene triplication
7	α2 Cod 142	Hb Koya Dora (A>C)
8	α2 Poly A-1	Saudi Type (AATAAA>AATAAG)
9	--FIL	Gene deletion (Double)
10	α1 cd 14	G>A
11	α1 cd 59	Hb Adana (G>A)
12	α2 Poly A-2	Turkish Type (AATAAA>AATGAA)
13	α2 Cod 142	Hb Icaria (T>A)
14	α2 init.cd	ATG>ACG
15	--THAI	Gene deletion (Double)
16	--SEA	Gene deletion (Double)
17	α2 cd 125	Hb Quong Sze (T>C)
18	α2 cd 142	Hb Constant Spring (T>C)
19	α2 cd 19	-G
20	α2 cd 142	Hb Paksé (A>T)
21	α2 cd 59	G>A

Table 2. The primer pairs for PCR reactions to amplify the target genes

Name of Primer	Sequence of Primer (5'-3')
HBA1-F	CTGTCTCCTGCCGACAAGACC
HBA1-R	GGGGGGAGGCCCAAGGGGCAAGAA
HBA2-F	CTGTCTCCTGCC GACAAGACC
HBA2-R	GGGAGGCCCAGCGGGCAGGAGGAAC

reveal the profile of alpha thalassemia mutations in different regions of Turkey (6-11). However, from Antalya Province where is the most prevelant of beta thalassemia and abnormal hemoglobins including Hb Antalya (12-17), there was no a report for α-thalassemia mutations at molecular genetic level.

In present study, we aimed to determine the molecular types and profile of deletional and non-deletional mutations and the genotype–phenotype correlation in common and rare mutations among α-thalassemia patients in the Antalya Province of Southern Turkey. We investigated alpha thalassemia mutations among suspected carriers who were send to our Genetic Laboratory from the Hematology Clinic Service at Akdeniz University and the Association of Mediterranean Blood Disease (AKHAV) in Antalya, Turkey.

Materials and Methods

A total of 250 potential carriers for alpha gene mutations were studied in three steps. They were identified as anemia with microcytic hypochromic. Their MCV (mean cell volume) was <80.0 fL. MCH (mean cell hemoglobin) was <27.0 pg and all of them had normal levels of Hb A2. Each patient signed an informed consent as a volunteer. Following the DNA isolation from peripheral blood (18), patients' DNAs were screened for the mutations of α-globin genes in two steps of molecular genetic analyses.

Firstly, the samples were screened for the known 21 mutations of alpha globin genes using reverse dot blot hybridization (RDBH) based with multiplex-PCR according to the commercial protocol (Alpha-globin StripAssay; ViennaLab Diagnostics, Vienna, Austria). These mutations are given in Table 1.

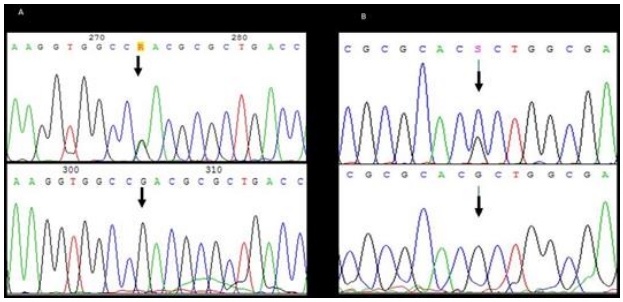

Fig. 1. Electropherograms of the two rare alpha globin mutations: A) HBA2:c.193 G>A (Hb GWaimanalo), B) HBA2:c.64 G>C (Hb Fontainebleau)

After the RDBH analysis, the patients who were with positive and negative mutation by RDBH were confirmed by MLPA method. This method was carried out using the commercial kit (MRC-Holland, the Netherlands). The MLPA assay was used for randomly selected 10 positive and 10 negative of patient samples analyzed by RDBH strip. The MLPA method was applied as described before (19,20).

Then, the samples without mutation by MLPA screening were sequenced by Sanger's sequencing using primers were designed according to reference sequences of HBA1 and HBA2 genes. Firstly, PCR was carried out for HBA1 and HBA2 genes using primer pairs (Table 2) in the PCR conditions as 95 oC for 60 sec, 56 oC for 45 sec, 72 oC for 90 sec 30 cycles, in 1.5 mM MgCl2, each primer as 10 pmol, 100 ng tamplete DNA in 25 ul final volume. Ethics Committee approved our study at Medical Faculty, Akdeniz University.

Results

In our study, eight different alpha globin gene mutations were determined in 112 (44.8%) patients. There was no mutation in the remaining 138 individuals of 250 participants (Table 3 and Table 4). The - $\alpha^{3.7}/\alpha\alpha$ was found as the most common genotype with 28.4%. The $-^{MED}/\alpha\alpha$ genotype was second common genotype with 7.0%. The most common mutation was the – $\alpha^{3.7}$ allele (73.2%) found in 123 alpha genes (Table 3). The – $\alpha^{20.5}$ (13.0%) and $-^{MED}$ (6.5%) were second and third most frequent mutations, respectively. The remaining frequent mutations were –FIL (2.4%), Hb Adana (2.4%). The 97.5 % of mutations consisted of these five mutations. The - $\alpha^{3.7}$ /-$(\alpha)^{20.5}$ genotype was found in three patients who had Hb H disease (1.2%) (Table 4).

From the results of the RDBH assay, 10 positive mutations (–MED, -$(\alpha)^{20.5}$ and - $\alpha^{3.7}$) and 10 negative mutations were randomly selected samples from cases. The RDBH assay results were confirmed by MLPA assay. All of the results were same with previous the RDBH assay results.

Of 21 common and known mutations on the strip, fifteen (-α 4.2, α2 IVS1 5nt deletion, α2 Hb Koya Dora (codon 142 (A>C)), α2 polyA1 (Saudi type), α1 codon 14 (G>A), α2 polyA2, Hb Icaria (α2 codon 142 (T>A)), α2 initiation codon ATG > ACG, -SEA, -THAI, Hb Constant Spring, α2 codon 19(-G), α2 codon 59 (G>A), Hb Paksé, α2 codon 125(T>C)) were not found in 140 individuals. These 140 subjects without mutation by strip assay based revers dot blot hybridization were sequenced. In addition, two rare mutations, α2 codon 64 (G>C) (Hb Fontainebleau) and α2 codon 193 (G>A) (Hb G-Waimanalo), were determined by DNA sequencing firstly in Antalya population, Turkey (21) (Figure 1). No alpha globin mutation was detected by both RDBH, the MLPA and DNA sequencing in remaining 138 individuals.

Discussion

According to World Health Organization (WHO), one fifth of the world's population are carriers of alpha thalassemia (22). The Middle Eastern and the Mediterranean are geographies where alpha thalassemia is concentrated. In there, the frequency of carriers increases up to 40%. (1,4). Turkey is a located both as a bridge between Asia, Africa, and Europe and in a geography with a high prevalence of hemoglobin disorders. The Turkish population made up of population with different ethnic origin. Therefore, the prevalence and profile of alpha globin gene mutations in every regions of the country need to be determined. In 2003, The Ministry of Health has started to screen the hemoglobinopathies with named as National Hemoglobinopathy Prevention Program in provinces with a high prevalence. With the start of this program, the screening rate of couples increased from 30% to 86% within 10 years (between 2003-2013) (23). The screening program was started with beta thalassemia and sickle cell anemia. The mutations of beta-thalassemia were well characterized by this program in Antalya as well as in the general of Turkey (12-14). However, studies on mutations of alpha globin genes were not parallely initiated to beta thalassemia. Firstly, in Antalya, carrier detection of the alpha thalassemia was done at peripheral blood level among newborns (24). Then, mutations in alpha genes have been identified in molecular genetic techniques in different regions of our country (8-11). Although, from Antalya Province where is the most prevelant of beta thalassemia (13) and abnormal hemoglobins including Hb Antalya (14), but the types and frequencies of mutations of alpha globin genes have never before been investigated.

Table 3. The numbers and frequencies of mutated alpha globin gene alleles in Antalya Province,Turkey

Mutation type	Numbers of chromosome affected	Allele (%)
- α 3.7	90	73.2
-(α) 20.5	16	13.0
--MEDII	8	6.5
--FIL	3	2.4
Hb Adana	3	2.4
ααα anti 3.7	1	0.8
Hb G Waimanalo	1	0.8
Hb Fontanebleau	1	0.8
Total	123	100.0

Table 4. The genotype frequencies, phenotypes, and mutation types in the patients studied in Antalya Province

Genotype	Mutation	Phenotype	N (%)
- α3.7 / αα	Deletion	Silent carrier	71 (28.4)
- α 3.7 /- α 3.7	Deletion	Alpha-thal trait	8 (3.2)
αα /-(α) 20.5	Deletion	Alpha-thal trait	13 (5.2)
- α3.7 / -(α) 20.5	Deletion	HbH	3 (1.2)
--MED/αα	Deletion	Alpha-thal trait	8 (7.0)
--FIL/αα	Deletion	Alpha-thal trait	3 (1.2)
ααAdana / αα	Non-deletion	Silent carrier	3 (1.2)
αanti-3.7α/αα	Triplication		1 (0.4)
αα / α Codon 64 α (Hb Fontanebleau)	Non-deletion	Silent carrier	1 (0.4)
αα / α Codon 193 α (Hb G Waimanalo)	Non-deletion	Silent carrier	1 (0.4)
ND Mutation*	ND*		138 (55.2)
Total			250 (100.0)

There was no a report for α-thalassemia mutations at molecular genetic level for Antalya population. In present study, - α $^{3.7}$ deletional mutation (73.2%) was the most prevalent α-globin gene mutation in Antalya. However, in the study with only strip assay, prevalence of the – α $^{3.7}$ deletion has been found lower (63.3%) in Adana (9), at the eastern of Turkey. In addition to – α $^{3.7}$ deletion, seven different mutations of alpha globin genes were found in our study. But the −4.2 deletion was not found in our investigation, although its prevalence was found as 1% in Adana that is near to Antalya (9). Furthermore, in the western of Turkey, the −4.2 deletion has not been found in İzmir province (10). It shows us to the fact that the frequency of the −4.2 deletion is decreasing from east to the west. Of the mutation detected 112 patients, three patients with the - α $^{3.7}$/-(α) $^{20.5}$ genotype Hb were found to have H disease (1.2%). However, the - α $^{3.7}$/-(α) $^{20.5}$ genotype was found in 10 (28.6%) of choosen 35 Hb H patients by Unal S et al. (11). In the current study, both of these alleles that caused the Hb H disease were the most prevalent mutations, - α$^{3.7}$ and -(α)$^{20.5}$ with the frequencies of 73.2% and 13.0%, respectively.

In our study, three different point mutations (non-deletional) were found named as the Hb Adana, Hb G Waimanalo, and Hb Fontanebleau variants. Of these, the most frequent variant was Hb Adana with 2.4% in the current investigation. All of them were the single allele mutation of 4 globin genes. Contrary to this, Bozdogan et al. reported the Hb Adana with 0.6% of the frequency in the 450 patients (9). -MED mutation is known as the most common mutation in the Turkish population (24). This mutation was seen as the third common mutation in both our and Unal S et al's study (11). In the alpha thalassemia study conducted with cord blood in the newborn group in Adana, the carrier rate was found to be 2.9% (25). Contrary to this, Canatan et al. found as 6.3% in Antalya region using the same method (26). In the another study, 206 patients had been studied and the 95 (46.1%) of patients have had 14 different

mutations in Ankara(27). In our study, 140 participants did not have any of the known 21 mutations on the strip. After the RDBH analysis, in the second step of our study, 10 patients with positive mutation and 10 patients with negative mutation obtained by RDBH were confirmed by MLPA method. All results were found in agreement. Then thirdly, the140 subjects that have no mutation by strip assay based revers dot blot hybridization were sequenced. After the sequencing, two rare mutations, α2 codon 64 (G>C) (Hb Fontainebleau) and α2 codon 193 (G>A) (Hb G-Waimanalo) were determined by DNA sequencing firstly in Antalya population, Turkey (19). No alpha globin mutation was detected by both revers dot blot hybridization and sequencing in remaining 138 individuals. These results shows that DNA sequencing should be used to determine the non-deletional mutations in screening of the alpha genes. In current study, we found the frequency of alpha-thalassemia as 44.8% in chosen patients with anemia and low blood values using molecular genetic techniques such as RDBH, MLPA and DNA sequencing in Antalya Province. Remaining patients with anemia and low blood values will be reviewed both clinically and laboratory for realistic diagnosis of alpha-thalassemia.

In conclusion, both beta and alpha thalassemia constitute as a socio-health problem in Antalya, Turkey as well as in the worldwide. This study has documented eight different alpha thalassemia alleles at the molecular genetic level in Antalya Province. We suggest that the analytical approaches such as carrier detecting, prenatal testing and giving the genetic information at premarital period should be planned in hemoglobinopathy management. Also, it need both clinically and technically to be screening of the alpha thalassemia mutations using complementary molecular genetic techniques in the specific regions.

References

1. Weatherall DJ, Clegg DJ. Inherited haemoglobin disorders: an increasing global health problem. Bull World Health Organ 2001; 79: 704-712.
2. Higgs DR, Vickers MA, Wilkie OA, Pretorius IM, Jarman AP, Weatherall DJ. A review of the molecular genetics of the human alpha-globin gene cluster. Blood 1989; 73: 1081-1104.
3. Kan YW. Molecular pathology of a-thalassemia Ann N Y Acad Sci 1985; 445: 28-36.
4. Vichinsky EP Clinical manifestiations of α-thalassemia. Cold Spring Harb Perpect Med 2013; 3: 1-10.
5. Keser I. Genetics of Hemoglobinopathies. In: Arapgirlioglu H, Atik A, Elliott RL, Turgeon E (eds). Researches on Science and Art in 21st Century Turkey. Ankara: Gece Publishing 2017; 2929-2937.
6. Ozsoylu Ş, Malik SA. Incidance of alpha thalassemia in Turkey. Turk J Pediatr 1982; 24: 235-244.
7. Altay Ç. The frequency and distribution pattern of alpha thalassemia mutation in Turkey. Turk J Hematol 2002; 19: 309-315.
8. Celik MM, Gunesacar R, Oktay G, Duran GG, Kaya H. Spectrum of α-thalassemia mutations including first observation of -(FIL) deletion in Hatay Province Turkey. Blood Cells Mol Dis 2013; 51: 27-30.
9. Bozdogan ST, Yuregir OO, Buyukkurt N, Aslan H, Ozdemir ZC, Gambin T. α - Thalassemia Mutations in Adana Province, Southern Turkey: Genotype-Phenotype Correlation. Indian J Hematol Blood Transfus 2015; 31: 223-228.
10. Onay H, Aykut A, Karaca E, Durmaz A, Solmaz AE, Çoğulu Ö, et al. Molecular spectrum of α-globin gene mutations in the Aegean region of Turkey: first observation of three α-globin gene mutations in the Turkish population. Int J Hematol 2015; 102: 1-6.
11. Unal Ş, Gumruk F. The hematological and molecular spectrum of alpha thalassemias in Turkey: Hacettepe Experience. Turk J Hematol 2015; 32: 136-143.
12. Keser I, Guzeloglu-Kayisli O, Yesilipek A, Ozes ON, Lüleci G. Hb Antalya [Codons 3-5 (Leu-Thr-Pro-Ser-Asp-Ser)]: A new unstable variant leading to chronic microcytic anemia and high HbA2, Hemoglobin 2001; 25: 369-373.
13. Keser I, Sanlıoglu AD, Manguoglu E, Guzeloglu Kayisli O, Nal N, Sargin F, et al. Molecular analysis of beta-thalassemia and sickle cell anemia in Antalya. Acta Haematol 2004; 111: 205-210.
14. Keser I, Yesilipek A, Canatan D, Lüleci G. Abnormal hemoglobins associated with the beta-globin gene in Antalya province, Turkey. Turk J Med Sci 2010; 40: 127-131.
15. Bilgen T, Arikan Y, Canatan D, Yeşilipek A, Keser I. The Association between Intragenic SNP Haplotypes and Mutations of the Beta Globin Gene in a Turkish Population. Blood Cells Molecules and Diseases 2011; 46: 226-229.
16. Bilgen T, Canatan D, Arikan Y, Yeşilipek A, Keser I. The effect of HBB:c.*+96T>C (3'UTR +1570 T>C) on mild beta-thalassemia intermedia phenotype. Turkish J Hematol 2011; 28: 219-222.

17. Bilgen T, Clark OA, Ozturk Z, Yeşilipek A, Keser I. Gap-PCR screening for common large deletional mutations of beta-globin gene cluster revealed a higher prevalence of the Turkish inv/del (deltabeta)o mutation in Antalya. Turk J Haematol 2016; 33: 107-111.
18. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 1988; 16: 1215.
19. White SJ, Vink GR, Kriek M, Wuyts W, Schouten J, Bakker B, et al. Two-color multiplex ligation-dependent probe amplification: detecting genomic rearrangements in hereditary multiple exostoses. Hum Mutat 2004; 24: 86-92.
20. Schouten JP, McElgunn CJ, Waaijer R, Zwijnenburg D, Diepvens F, Pals G. Relative quantification of 40 nucleic acid sequences by multiplex ligationdependent probe amplification. Nucleic Acids Res 2002; 30: 57.
21. Canatan D, Bilgen T, Ciftci V, Yazıcı G, Delibaş S, Keser I. First Observation of Hemoglobin G-Waimanalo and Hemoglobin Fontainebleau Cases in the Turkish Population. Turk J Hematol 2016; 3: 71-83.
22. Modell B, Darlison M. Global epidemiology of haemoglobin disorders and derived service indicators. Bull World Health Organ 2008; 86: 480-487.
23. Canatan D. Thalassemias and hemoglobinopathies in Turkey. Hemoglobin 2014; 38: 305-307.
24. Huisman THJ, Carver MFH, Baysal E. A Syllabus of Thalassemia Mutations. Augusta, GA, USA, Sickle Cell Anemia Foundation 1997.
25. Kılınç Y, Kumi M, Gürgey A, Altay Ç. Investigation of the frequency of β-thalassemia, glucose-6-phosphate dehydrogenase enzyme deficiency and Hb S with cord blood study in babies born in Adana Region. Doğa 1986; 10: 162-167 (in Turkish).
26. Canatan D, Oguz N, Guvendik I, Yildirim S. The Incidence of Alpha-Thalassemia in Antalya- Turkey. Turk J Hematol 2002; 19: 433-434.
27. Karakaş Z, Koç B, Temurhan S, Elgün T, Karaman S, Asker G, et al. Evaluation of Alpha-Thalassemia Mutations in Cases with Hypochromic Microcytic Anemia: The Istanbul Perspective. Turk J Hematol 2015; 32: 344-350.