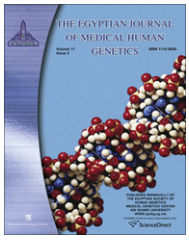
The Egyptian Journal of Medical Human Genetics (2011) **12**, 59–62

Ain Shams University

**The Egyptian Journal of Medical Human Genetics**

www.ejmhg.eg.net
www.sciencedirect.com



ORIGINAL ARTICLE

# Hemoglobin alpha 2 gene +861 G > A polymorphism in Turkish population

**Dilay Ciglidag Dungul [a], Hilal Ozdag [a], Nejat Akar [b,*]**

[a] *Genomics Unit of Central Laboratory, Ankara University Biotechnology Institute, Ankara, Turkey*
[b] *Department of Pediatric Molecular Genetics, Ankara University, Ankara, Turkey*

Received 4 October 2010; accepted 2 January 2011

**KEYWORDS**

Alpha thalassemia;
*HBA1*;
*HBA2*;
Polymorphism

**Abstract** Thalassemia is an inherited blood disorder which is divided into two groups: alpha and beta. *HBA1* and *HBA2* are the two genes associated with alpha thalassemia. The aim of this study is to investigate abnormal hemoglobin variants of alpha globin gene in healthy abnormal hemoglobin carrying individuals with intact beta globin gene. DNA was extracted from peripheral blood samples of seven healthy carrier individuals who have abnormal hemoglobin variants and 16 control individuals from Turkey. Complete coding and intronic sequences of *HBA1* and *HBA2* genes were amplified by polymerase chain reaction (PCR) and PCR products of *HBA1* and *HBA2* were sequenced. We were unable to find any base change in our carrier group in the *HBA1* gene. We have observed an A/G polymorphism in the downstream untranslated region (+861 G > A) of the *HBA2* gene. Our study showed that 14.29% (1/7 carriers) of the carrier group and 37.50% (6/16 controls) of the control group were heterozygous for the +861 G > A polymorphism. The distribution of allele frequencies and genotypes of *HBA2* between carrier and control samples were analyzed and it is seen that the distribution of allele frequencies and that of genotypes were not statistically significant between carrier and control samples (*P*-value = 0.4131, *P*-value = 0.366, respectively). *HBA2* +861 G > A nucleotide substitution is a neutral polymorphism previously reported in other populations. This is the first report in Turkish population.



* Corresponding author. Tel.: +90 312 595 63 48; fax: +90 312 320 14 33.
E-mail address: Nejat.Akar@medicine.ankara.edu.tr (N. Akar).



Peer review under responsibility of Ain Shams University.
doi:10.1016/j.ejmhg.2011.02.005

ELSEVIER **Production and hosting by Elsevier**

## 1. Introduction

Thalassemia is an autosomal recessive genetic blood disorder that affects a patient's ability to produce hemoglobin, resulting in anemia. The disease is common in Mediterranean, Middle Eastern and Southeast Asian regions [1]. The thalassemias are classified according to the type of globin polypeptide chain that is underproduced. The alpha chain is involved in alpha thalassemia and the beta chain is affected in beta thalassemia. Alpha thalassemia is caused by mutations in the alpha chain of the hemoglobin molecule. There are two carrier states of alpha

thalassemia. Silent carrier is characterized by the loss of only one gene whereas the second carrier state is characterized by the loss of two genes producing a condition with small red blood cells and, at most, a mild anemia. Besides there are two clinically significant forms of alpha thalassemia syndrome [2,3]. These are Hemoglobin H (HbH) disease and Hb Bart hydrops fetalis.

More than 20 different deletional alpha thalassemia mutations and 32 different nondeletional alpha thalassemia mutations which include mutations affecting RNA translation/processing, mutations in the coding sequences and mutations in poly A have been reported worldwide [4].

Although alpha thalassemia is rare, more frequently hemoglobin H disease has been observed in Turkey. The incidence of alpha thalassemia is about 3% in the Çukurova region located at the south of Turkey [5–7].

*HBA1*, the gene encoding hemoglobin alpha 1, and *HBA2*, the gene encoding hemoglobin alpha 2, are the two genes associated with alpha thalassemia. *HBA1* and *HBA2* are duplicated and localized to the telomeric region of chromosome 16p within a cluster that includes an embryonic α-like gene and three pseudogenes. *HBA1* and *HBA2* consist of three exons. DNA sequence analysis of human *HBA1* and *HBA2* shows that their coding sequences are identical. These genes differ slightly over the 5′ untranslated regions and the introns, but they differ significantly over the 3′ untranslated regions. These genes show overall sequence homology >96% [8,9]. Because of the repetitive nature of this gene cluster, the alpha globin genes are prone to deletion. Alpha thalassemias result from deletions of each of the alpha genes as well as deletions of both of them. Some nondeletional alpha thalassemias have also been reported [8]. Most of the mutations in alpha globin genes are deletions and point and regulatory region mutations are relatively rare.

According to Human Gene Mutation Database (HGMD) more than 48 different mutations (missense/nonsense, splicing, regulatory, small deletion, small indel, gross deletion and complex rearrangement) have been reported for the *HBA2* gene and 28 different mutations have been reported for *HBA1* gene for alpha thalassemia phenotype. PCR-based methods provide a rapid diagnosis and may be used to detect deletions of the gene *HBA1* and *HBA2*. Sequence analysis can be used to identify less frequent point mutations or sequence variation, in the coding sequences of *HBA1* and *HBA2* [10].

Several deletional and nondeletional alpha thalassemia determinants are present in Turkish populations. Five gene deletions (alpha1 (MED I: −17.4 kb, MED II: −26.5 and −20.5 kb, alpha 2 (−3.7 and −4.2 kb), two different Poly A mutations (nondeletional alpha thalassemia mutations on *HBA2* gene which are specific to Mediterranean countries PA1: AATAAA → AATAAG ve PA2: AATAAA → AATGAA), a few base pair deletion (−5 nt (-TGAGG)) on the *HBA2* gene and a point mutation (Hb Adana, CD 59: GGC → GAC; Gly → Asp) on the *HBA1* gene were reported in Turkey [6,11]. Hb Adana and Poly A (PA2) mutations are firstly defined in Turkey [12].

In this study we investigated abnormal hemoglobin variants of alpha globin gene in healthy abnormal hemoglobin carrying individuals with intact beta globin gene. For this purpose, we sequenced alpha globin genes and have found +861 G>A polymorphism in hemoglobin alpha 2 globin gene.

## 2. Subjects and methods

Seven healthy abnormal hemoglobin carrier individuals and 16 control individuals were included in this study. None of the carriers have hemoglobin variant in beta globin gene. Control individuals are healthy, with normal complete blood count. Control samples were included in this study to display relationship between the alleles (G and A) and the disease.

Written informed consent is obtained from all the patients and controls.

DNA was extracted from peripheral blood samples according to standard phenol–chloroform protocol. Complete coding and intronic sequences of *HBA1* and *HBA2* genes were amplified by polymerase chain reaction (PCR). The sequences of the PCR primers are listed in Table 1.

We have amplified *HBA* genes as a long single amplicon (*HBA1* 725 bp, *HBA2* 735 bp) using a common forward primer and gene specific reverse primer (Fig. 1). We have used

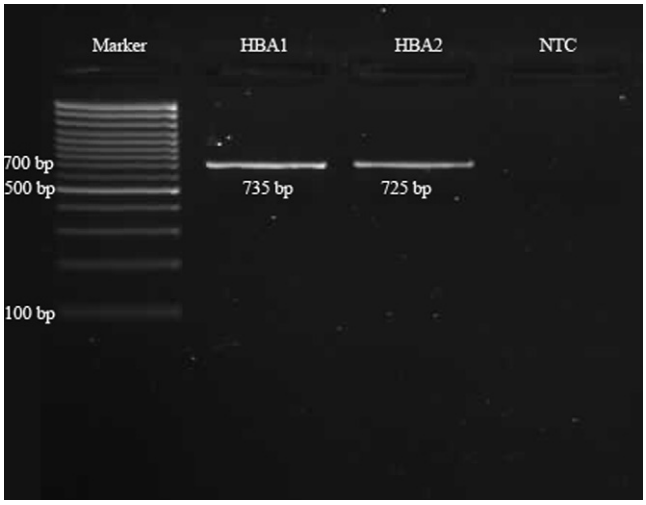



**Figure 1** PCR amplification of *HBA1* and *HBA2* genes. Lane 1: 100 bp marker, lane 2: *HBA1* amplification (735 bp), lane 3: *HBA2* amplification (725 bp), lane 4: non-template control (NTC).

**Table 1** Primers designed for the amplification *HBA1* and *HBA2* genes.

| Primer | Length of the amplicon | Loci | Sequence (5′–3′) of the primers |
|---|---|---|---|
| Forward primer | | *HBA1* and *HBA2* | CTGTCTCCTGCCGACAAGACC |
| Reverse primer | 725 bp (from 44th base to 778th base) | *HBA1* | GGGGGGAGGCCCAAGGGGCAAGAA |
| Reverse primer | 735 bp (from 73th base to 798th base) | *HBA2* | GGGAGGCCCAGCGGGCAGGAGGAAC |

**Table 2** Primers designed for the sequence reaction of *HBA1* and *HBA2* genes.

| Primer | Loci | Sequence (5′–3′) of the primers |
|---|---|---|
| Nested primer 1F | *HBA1*, *HBA2* | CCCCAAGCATAAACCCTG |
| Nested primer 1R | *HBA1*, *HBA2* | GGGGAAGGACAGGAACATC |
| Nested primer 2F | *HBA1*, *HBA2* | AGACTCAGAGAGAACCCACC |
| Nested primer 2R | *HBA1*, *HBA2* | GCTCACCTTGAAGTTGACC |
| Nested primer 3F | *HBA1*, *HBA2* | ACCACCAAGACCTACTTCC |
| Nested primer 3R | *HBA1*, *HBA2* | AACGGTATTTGGAGGTCAG |
| Nested primer 4F | *HBA1*, *HBA2* | GGTCAACTTCAAGGTGAGC |
| Nested primer 4R | *HBA1* | ACTTTATTCAAAGACCACGG |
| Nested primer 5F | *HBA1* | AGTTCCTGGCTTCTGTGAG |
| Nested primer 5F/2 | *HBA2* | TCCAAATACCGTTAAGCTG |
| Nested primer 5R | *HBA1* | GAAAGAGCAAATGCATCCT |

nested primers to sequence both genes. The sequences of the nested primers are listed in Table 2.

PCR products of *HBA1* and *HBA2* were sequenced using Beckman DTCS Quick Start Sequencing Kit (Beckman Coulter CEQ 8000 DNA Sequencer, USA) according to the manufacturer's instructions. Sequencing reactions of *HBA1* and *HBA2* were carried out using various primers which was given above. Sequence analysis was performed with SEQUENCHER (Gene Codes Corporation, USA) sequence analysis software.

The distribution of allele frequencies and genotypes of *HBA2* between carrier and control samples were compared with Fisher's exact test. On the other hand, Hardy–Weinberg equilibrium (HWE) of genotypes frequencies was tested through Fisher's exact test since some of the expected counts are less than 5. All tests were performed by statistical software R (version 2.10.1). *P*-values less than 0.05 were considered as statistically significant.

## 3. Results

In our study, seven healthy abnormal hemoglobin carriers without any mutation/polymorphism in the beta globin gene are used as the case group. We have sequenced the alpha globin genes of these seven individuals and have found +861 G > A polymorphism in hemoglobin alpha 2 globin gene. We than have screened this polymorphism in 16 healthy individuals as the control group.

We were unable to find any base change in our carrier group in the *HBA1* gene. The observed polymorphism, +861 G > A, is located (AGCCTGTGTGTGCCTGGGTTC TCTCT(A/G)TCCCGGAATGTGCCAACAATGG) in the downstream untranslated region of the *HBA2* gene (Figs. 1 and 2). We have detected this variation only in one out of seven carrier and six out of sixteen control samples.

It is observed that while the frequency of G allele in carrier and control samples were 0.93% and 0.81%, respectively, the frequency of A allele was 0.07% in carrier samples and 0.19% in control samples (Table 3). The distribution of G and A alleles was found to be statistically insignificant between carrier and control samples (*P*-value = 0.4131).

Similarly, our results showed that while six out of seven healthy carrier individuals genotype was homozygote (GG) and one carrier's genotype was heterozygote (AG), the genotypes of 10 of the 16 control sample individuals were homozygote (GG) and those of six of them were heterozygote (AG) (Table 4). The distribution of GG and AG genotypes was statistically insignificant between carrier and control samples (*P*-value = 0.366). The distribution of the genotypes frequencies

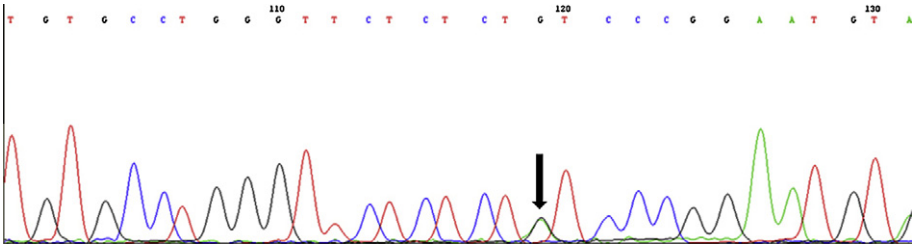



**Figure 2** Electroferogram of the DNA sequence, showing a G to A polymorphism in the downstream untranslated region of the *HBA2* gene.

**Table 3** The distribution of +861 G > A *HBA2* polymorphism allele frequencies in carrier and control samples.

| Allele | No. of case (%) (*n* = 7) | No. of control (%) (*n* = 16) | *P*-value |
|---|---|---|---|
| G | 13 (0.93%) | 26 (0.81%) | 0.4131 |
| A | 1 (0.07%) | 6 (0.19%) | |

**Table 4** The distribution of +861 G > A *HBA2* polymorphism genotypes in carrier and control samples.

| Genotype | No. of case (%) ($n$ = 7) | No. of control (%) ($n$ = 16) | $P$-value |
|---|---|---|---|
| Homozygote (GG) | 6 (85.71%) | 10 (62.50%) | 0.366 |
| Heterozygote (AG) | 1 (14.29%) | 6 (37.50%) | |

was found to be consistent with Hardy–Weinberg equilibrium ($P$-value > 0.05).

## 4. Discussion

Substitutions causing regulatory abnormalities are logged in with 30 nucleotides flanking the site of the mutation on both sides known as regulatory mutation (HGMD). Mutations in Poly A suppress protein synthesis because of the immature mRNA or decrease protein level due to the short lived mature mRNA. The location of this substitution we have found is 48 nucleotide relative to poly A signal (+861 G > A).

Lacerra et al. defined this (+861 G > A) variation as a common nonpathogenic sequence variation 9. Passarello et al. showed occurrence of the two single point mutations that are characteristic of Hb Caserta and Hb Sun Prairie, and the +861 G > A polymorphic site in the homozygous state and Hb Southern Italy variant, mutation in *HBA2* (130 G → C) is always associated with another mutation 26 G → A and with the polymorphic site *HBA2* +861 [13,14]. Hb Constant Spring was confirmed to be associated *in cis* with the neutral SNP *HBA2* +861 G > A [14]. +861 G > A in *HBA2* gene defined as a common neutral polymorphism in 26 of the 83 Sicilian individuals [15].

The ratio of single base mutations in regulatory unit is lower than splicing, nonsense and missense mutations and higher than silent and frame shift mutations. Mutations in the 5′ UTR can affect the binding of trans-acting factors that cause increment or decrement of translation efficiency [16,17].

In our study, during screening of abnormal hemoglobin variants at the *HBA2* gene, we detected a previously reported +861 A > G substitution located 48 nucleotide relative to poly A signal in *HBA2* gene. To conclude, although this neutral polymorphism was reported in other populations, this is the first report from Turkish population.

## Conflicts of interest

The authors of this paper declare that they have no conflict of interests.

## Acknowledgement

We thank Gul Inan, M.Sc., Department of Statistics, METU, for helping in statistical analysis.

## References

[1] Alan RC, Galanello R, Dudley JP, Melody JC, Elliott V. Thalassemia. Hematology 2004;1:14–34.

[2] Ribeiro DM, Sonati MF. Regulation of human alpha-globin gene expression and alpha-thalassemia. Genet Mol Res 2008;7(4):1045–53.

[3] Çürük MA, Kılınç Y, Evrüke C, Özgünen FT, Aksoy K, Yüreğir GT. Prenatal diagnosis of Hb H disease caused by alpha homozygosity for the alpha 2 Poly A (AATAAA-AAATAAG) mutation. Hemoglobin 2001;25(2):255–8.

[4] Huisman THJ, Carver MFH, Baysal E. A syllabus of thalassemia mutations. Augusta, GA, USA: The Sickle Cell Anemia Foundation; 1997.

[5] Canatan D, Oğuz N, Güvendik İ, Yıldırım S. The incidence of alpha-thalassemia in Antalya-Turkey. Turk J Hematol 2002;19(3):433–4.

[6] Çürük MA, Genç A, Huseynova P, Zeren F, Aksoy K. Genotypes of alpha thalassemia and HbH disease in Çukurova. Turkiye Klinikleri J Pediatr Sci 2007;3(10):17–23.

[7] Kılınç Y, Kümi M, Gürgey A, Altay Ç. Adana bölgesinde doğan bebeklerde kordon kanı çalışması ile alfa talasemi, G6PD enzim eksikliği ve HbS sıklığının araştırılması. Doğa Tr Tıp ve Ecz D 1986;10(2):162.

[8] Higgs DR, Vickers MA, Wilkie AO, Pretorius IM, Jarman AP, Weatherall DJ. A review of the molecular genetics of the human alpha-globin gene cluster. Blood 1989;73:1081–104.

[9] Lacerra G, Fiorito M, Musollino G, Noce FD, Esposito M, Nigro V, Gaudiano C, Carestia C. Sequence variations of the a-globin genes: scanning of high CG content genes with DHPLC and DG-DGGE. Hum Mutat 2004;24:338–49.

[10] Galanello R, Sollaino C, Paglietti E, Barella S, Perra C, Doneddu I, Pirroni MG, Maccioni L, Cao A. α-Thalassemia carrier identification by DNA analysis in the screening for thalassemia. Am J Hematol 1998;59:273–8.

[11] Öner C, Gürgey A, Öner R, Balkan H, Gümrük F, Baysal E, Altay C. The molecular basis of Hb H disease in Turkey. Hemoglobin 1997;21:41–51.

[12] Yüregir GT, Aksoy K, Çürük MA, Dikmen N, Fei YJ, Baysal E, Huisman THJ. HbH disease in a Turkish family resulting from the interaction of a deletional α-thalassemia-1 and a newly discovered poly A mutation. Br J Haematol 1992;80:527–32.

[13] Passarello C, Giambona A, Prossomariti L, Ammirabile M, Pucci P, Renda D, Pagano L, Maggio A. Hb southern Italy: coexistence of two missense mutations (the Hb Sun Prairie α2 130 Ala → Pro and Hb Caserta α2 26 Ala → Thr) in a single HBA2 gene. Br J Haematol 2008;143:138–42.

[14] Lacerra G, Musollino G. Genotyping for known Mediterranean α-thalassemia point mutations using a multiplex amplification refractory mutation system. Haematologica 2007;92:254–5.

[15] Guida V, Colosimo A, Fichera M, Lombardo T, Rgcit L, Dallayıccola B. Hematologic and molecular characterization of a Sicilian cohort of α thalassemia carriers. Haematologica 2006;91:409–10.

[16] Niranjan V, Mahmood R, Saxena A, KalaiVani A. UTR mutation analysis. JCIB 2008;1:55–63.

[17] Chen JM, Ferec C, Cooper DN. A systematic analysis of disease-associated variants in the 3′ regulatory regions of human protein-coding genes I: general principles and overview. Hum Genet 2006;120(1):1–21.