

# $\beta$ -Globin chain abnormalities with coexisting $\alpha$ -thalassemia mutations

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## Abstract

**Introduction:** The frequency of hemoglobinopathies is still high in Adana, the biggest city of the Cukurova Region that is located in the southern part of Turkey. Our aim was to identify the concomitant mutations in  $\alpha$ - and  $\beta$ -globin genes which lead to complex hemoglobinopathies and to establish an appropriate plan of action for each subject, particularly when prenatal diagnosis is necessary.

**Material and methods:** We studied the association between the  $\beta$ -globin gene and  $\alpha$ -thalassemia genotypes. The reverse hybridization technique was employed to perform molecular analysis, and the results were confirmed by amplification refractory mutation system (ARMS) or restriction fragment length polymorphism (RFLP) technique.

**Results:** We evaluated 36 adult subjects (28 female and 8 male; age range: 18-52 years) with concomitant mutations in their  $\alpha$ - and  $\beta$ -globin genes. The  $-\alpha^{3.7}/\alpha\alpha$  deletion was the commonest defect in the  $\alpha$ -chain as expected, followed by  $\alpha^{3.7}/-\alpha^{3.7}$  deletion. Twenty-five of 36 cases were sickle cell trait with coexisting  $\alpha$ -thalassemia, while seven Hb S/S patients had concurrent mutations in their  $\alpha$ -genes. The coexistence of  $\alpha^{\text{PolyA-2}}/\alpha\alpha$  with Hb A/D and with Hb S/D, which is very uncommon, was also detected. There was a subject with compound heterozygosity for  $\beta$ -globin chain ( $-\alpha^{3.7}/\alpha\alpha$  with IVS1.110/S), and also a case who had  $-\alpha^{3.7}/\alpha\alpha$  deletion with IVS1.110/A.

**Conclusions:** Although limited, our data suggest that it would be valuable to study coexisting  $\alpha$ -globin mutations in subjects with sickle cell disease or  $\beta$ -thalassemia trait during the screening programs for premarital couples, especially in populations with a high frequency of hemoglobinopathies.

**Key words:** a-globin gene, b-globin gene, mutation, S- $\alpha$ -thalassemia, sickle cell anemia.

## Introduction

The hemoglobin (Hb) molecule consists of two subunits controlled by genes in the  $\alpha$  gene cluster on chromosome 16 and two subunits derived from genes in the  $\beta$  gene cluster on chromosome 11 [1]. Abnormalities in Hb synthesis may cause two separate genetic disorders: the thalassemias, a group of syndromes associated with absent or decreased production of

particular globin chains; and abnormal hemoglobins that result from structural alterations in the Hb molecule, such as single and double amino acid substitution, amino acid deletions or insertions [2].

The thalassemias, one of the most widespread hereditary diseases worldwide, can be classified into two major groups according to the deficient globin chains:  $\alpha$ -thalassemia ( $\alpha$ -thal) is commonly encountered in Southeast Asia and Southern China, whereas  $\beta$ -thalassemia ( $\beta$ -thal) is more prevalent in the Mediterranean countries [3]. In Turkey, the frequency of  $\beta$ -thal carriers has been reported to be 4.3%; however, the incidence may be as high as 10% in particular areas [4, 5]. A recent survey conducted by our group revealed that the rate of occurrence for  $\alpha$ - and  $\beta$ -thal carriers in Adana is 7.5% [6] and 13.5% respectively (unpublished data).

The synthesis of structurally abnormal globin chains, on the other hand, causes abnormal hemoglobins, and the substitution of a single nucleotide for another is suggested to be the most common genetic alteration that results in hemoglobin variants [7]. Sickle hemoglobin (Hb S), for example, is caused by the replacement of glutamic acid by valine in the 6<sup>th</sup> codon of the  $\beta$ -globin chain, and sickle cell disease (SCD) affects millions throughout the world [8]. In Southern Turkey, particularly in Adana, SCD is still very common and is considered to be a significant public health concern with an incidence of the heterozygous Hb S of 8.2% [9]. Our group has recently found that the frequency of Hb S is approximately 6.4% in Adana (unpublished data).

Although the exact mechanism by which disorders of hemoglobin structure and production protect against malaria is unknown, it has long been known that Hb S and the thalassemias provide a survival advantage over people with normal hemoglobin in malaria-endemic regions. Therefore, the frequency of hemoglobinopathies is significantly increased around the world in those places where the malaria incidence is high [10, 11]. For instance, Adana is located on the Cukurova Plain, one of the malaria-endemic regions of Turkey, and has been reported to have Hb S and thalassemias with a high prevalence [12].

Both thalassemias and homozygous SCD represent considerable variations in their clinical presentations, especially when inherited together. The clinical and hematological manifestations of sickle cell anemia (SCA) have been found to be altered by the presence of  $\alpha$ - and  $\beta$ -thals [13, 14]. Moreover, several studies have shown that the coexistence of  $\alpha$ -thal with SCA can produce consequences that are beneficial in some complications but detrimental in others [15]. As an example, a concurrent  $\alpha$ -thal has been shown to reduce the concentration of Hb S and Hb S polymerization in patients with SCA [16]. Similarly, in thalassemia major or intermedia, reduction in the number of  $\alpha$ -globin genes can

ameliorate the disease phenotype. Thus, in order to design an appropriate treatment strategy for newly diagnosed patients, to detect atypical carriers in prenatal diagnosis, and to provide the most accurate guidance during genetic counseling, it seems to be valuable in clinical practice to recognize the complex phenotypes and the relationship between genotype and phenotype.

In this study, we retrospectively evaluated a total of 36 cases with concurrent  $\alpha$ - and  $\beta$ -globin gene mutations to describe the hematological and the molecular data resulting from the interaction between  $\alpha$ - and  $\beta$ -globin chain abnormalities in Adana, Turkey.

## Material and methods

Seyhan Hereditary Blood Disorders Center was established with the aim of providing genetic counseling for hemoglobinopathy carriers and conducting screening surveys in Adana. Thus, most of the subjects evaluated in this study were premarital couples or individuals with a complaint of anemia. Data from 36 adult individuals, consisting of 28 females and 8 males with an age range of 18-52 years, were analyzed.

In order to perform a routine screening protocol for hemoglobinopathies, 3 ml of blood samples in EDTA were obtained from all cases. A complete blood count and high performance liquid chromatography (HPLC) analysis were carried out to detect  $\alpha$ - and  $\beta$ -thal carriers and Hb variants as well, while reverse hybridization technique was employed to identify mutations in the  $\alpha$ - and  $\beta$ -globin genes.

After iron-deficient patients were eliminated from consideration, subjects with low mean corpuscular volume (MCV < 80 fl), mean cell hemoglobin (MCH < 25 pg), and mean cell hemoglobin concentration (MCHC < 30 g/dl) levels were evaluated in two groups: the ones with increased Hb A<sub>2</sub> level (> 3.7%) were further analyzed with regard to possible mutations in their  $\beta$ -globin chains. The second group consisted of subjects with normal/ decreased Hb A<sub>2</sub> levels (< 2.5%), and screened for mutations in the  $\alpha$ -globin gene. Subsequently, both groups were analyzed for mutations in the other globin chain.

To achieve molecular analysis, we isolated DNAs from leukocytes according to the procedure described by Poncz *et al.* [17]. We used the reverse hybridization method using a StripAssay (ViennaLab, Austria), and to confirm the results we used the amplification refractory mutation system (ARMS) or restriction fragment length polymorphism (RFLP) as a second molecular diagnostic method.

## Results

We found 36 subjects with a concomitant mutation in their  $\alpha$ - and  $\beta$ -globin genes.  $-\alpha^{3.7}/\alpha\alpha$  deletion

**Table I.** The coexistence of  $\alpha$ -thalassemia mutations with  $\beta$ -globin chain abnormalities

$\alpha$ -Thal mutations	$\beta$ -Globin chain mutations	Number
$-\alpha^{3.7}/\alpha\alpha$	HbA/S	16
$-\alpha^{3.7}/-\alpha^{3.7}$	HbA/S	6
$-\alpha^{3.7}/-\alpha^{3.7}$ MED	HbA/S	2
$-\alpha^{3.7}/\alpha\alpha$	HbS/S	2
$-\alpha^{3.7}/-\alpha^{3.7}$	HbS/S	3
$\alpha\alpha\alpha^{3.7}/\alpha\alpha$	HbS/S	2
$\alpha\alpha\alpha^{3.7}/\alpha\alpha$	HbA/S	1
$\alpha^{\text{PolyA-2}}/\alpha\alpha$	HbA/D	1
$\alpha^{\text{PolyA-2}}/\alpha\alpha$	HbS/D	1
$-\alpha^{3.7}/\alpha\alpha$	IVS1.110/S	1
$-\alpha^{3.7}/\alpha\alpha$	IVS1.110/A	1
<b>Total</b>		<b>36</b>

coexisting with Hb A/S was the most frequent form among our cases ( $n = 16$ ) followed by  $-\alpha^{3.7}/-\alpha^{3.7}$  deletion with Hb A/S ( $n = 6$ ). Two Hb A/S cases with  $-\alpha^{3.7}/-\alpha^{3.7}$  MED mutation in their  $\alpha$ -globin chains were also detected. There were a total of seven Hb S/S patients with coexisting  $-\alpha^{3.7}/\alpha\alpha$  ( $n = 2$ ),  $-\alpha^{3.7}/-\alpha^{3.7}$  deletions ( $n = 3$ ), and anti-3.7 gene triplication/ $\alpha\alpha$  ( $n = 2$ ), while a subject with sickle cell trait (Hb A/S) was also shown to have anti-3.7 gene triplication/ $\alpha\alpha$ .

Of two cases with  $\alpha^{\text{PolyA-2}}/\alpha\alpha$  mutation, one showed Hb A/D and another had Hb S/D genotype. We also identified a subject with compound heterozygosity for  $\beta$ -globin chain ( $-\alpha^{3.7}/\alpha\alpha$  deletion with IVS1.110/S), and  $-\alpha^{3.7}/\alpha\alpha$  deletion with IVS1.110/A (Table I).

According to the complete blood count (CBC) results, there was an inconsistency between MCV, MCH and MCHC values in our group. Although most of the subjects had low Hb, hematocrit (HCT) and MCV levels, as expected, the MCH and MCHC levels were found to be normal or elevated in some cases (Table II).

When we look at the sickle cell trait subjects with  $-\alpha^{3.7}/\alpha\alpha$  deletion, the MCV levels were found to be below 80 fl, except in two of 16 individuals. Surprisingly, all cases had a normal MCHC level. The MCV values in sickle cell trait with  $-\alpha^{3.7}/-\alpha^{3.7}$  were found to be lower compared to this group. Although there were only two Hb A/S subjects with  $-\alpha^{3.7}/-\alpha^{3.7}$  MED mutation in their  $\alpha$ -globin gene, the MCV and the MCH results obtained from these cases were the lowest. The hematologic index of the only Hb A/S case with anti-3.7 gene triplication was noted to be slightly decreased.

We had a total of seven homozygous SCD cases with co-inherited  $\alpha$ -thal in our group. Patients with one or two deletions or triplications in the

$\alpha$ -globin gene showed different results in terms of mean hemoglobin levels. The lowest hemoglobin values were obtained in SCD patients with  $-\alpha^{3.7}/\alpha\alpha$  deletion. Furthermore, we found no significant changes in the red cell indices of Hb A/D and Hb S/D cases who had coexisting  $\alpha^{\text{PolyA-2}}/\alpha\alpha$  mutation. Also, the mean hemoglobin level of a subject carrying an  $-\alpha^{3.7}/\alpha\alpha$  deletion with IVS1.110/S was detected to be decreased compared to those who had  $-\alpha^{3.7}/\alpha\alpha$  deletion with IVS1.110/A.

### Discussion

The thalassemias and abnormal hemoglobins constitute a group of the most common hereditary blood disorders in the world caused by quantitative or structural defects in hemoglobin synthesis [1, 2]. The foremost Hb disorder in Turkey, as in other Mediterranean countries, is stated to be  $\beta$ -thal followed by sickle cell disease, which occurs at the highest frequency in Adana province, one of the malaria-endemic areas in Turkey [18]. A number of factors, such as the wide ethnic variety, the unusual frequency of consanguineous marriages, and the high rate of birth, are presumed to be involved and contribute to the increased prevalence of hemoglobinopathies, as well as the co-inheritance of  $\alpha$ - and  $\beta$ -globin gene mutations in this area [4].

In this study, 36 subjects with various  $\alpha$ -thal mutations have been found to have concomitant deficiencies in their  $\beta$ -globin genes. In the Mediterranean countries, the predominant mutation of the  $\alpha$ -globin gene has been stated to be the  $-\alpha^{3.7}$  single gene deletion [3]. Consistent with this observation, we identified  $-\alpha^{3.7}$  deletions in 20 out of 36 cases. Sixteen subjects with  $-\alpha^{3.7}$  mutation were shown to have coexisting sickle cell trait (Hb A/S), while another two were homozygous SCD (Hb S/S). Moreover, two cases with  $-\alpha^{3.7}$  single gene deletion were detected to have co-inheritance with  $\beta$ -thal (IVS1.110/A), and one of them had additional sickle cell trait (IVS1.110/S).

Sankar *et al.* reported a study of 276 cases, and found that 33 of these individuals had  $-\alpha^{3.7}$  deletions (12%), seven of them with concurrent SS/S $\beta$ -thalassemia [19]. Similar results were published by Nava *et al.*, who found  $-\alpha^{3.7}$  deletions to be the most common mutations in the  $\alpha$ -globin chain, and observed  $-\alpha^{3.7}$  deletion and anti-3.7 gene triplication with the presence of the sickle cell trait in 17 and in three cases respectively [20].

In our study,  $-\alpha^{3.7}/-\alpha^{3.7}$  deletions were identified in nine subjects. While the coexisting sickle cell trait (Hb A/S) was defined in six of these cases, the remaining three cases were homozygous SCD. Our data indicated that  $-\alpha^{3.7}/-\alpha^{3.7}$  mutation caused decreased hematological results in cases with sickle cell trait, but had nearly no effect in homozygous SCD patients.

Table II. Hematological data in α- and β-globin gene mutations

No.	RBC [10 <sup>12</sup> /l]	Hgb [g/dl]	Hct [%]	MCV [fl]	MCH [pg]	MCHC [g/dl]	Type of mutation in β-globin chain	α-Thal genotype
1	4.91	10.8	32.4	65.9	21.9	33.3	HbA/S	-α <sup>3.7</sup> /αα
2	5.04	13.8	41.2	81.8	27.5	33.6	HbA/S	-α <sup>3.7</sup> /αα
3	4.63	14.5	36.8	79.5	31.3	39.4	HbA/S	-α <sup>3.7</sup> /αα
4	3.74	6.9	22.9	61.2	18.5	30.2	HbA/S	-α <sup>3.7</sup> /αα
5	4.90	13.0	35.7	72.8	26.5	36.4	HbA/S	-α <sup>3.7</sup> /αα
6	5.42	12.6	37.3	68.8	23.3	33.9	HbA/S	-α <sup>3.7</sup> /αα
7	5.26	13.4	42.3	80.3	25.4	31.7	HbA/S	-α <sup>3.7</sup> /αα
8	4.08	6.3	20.2	49.4	15.4	31.1	HbA/S	-α <sup>3.7</sup> /αα
9	4.44	10.9	32.8	73.8	24.5	33.2	HbA/S	-α <sup>3.7</sup> /αα
10	5.22	11.5	34.1	65.2	22.1	33.0	HbA/S	-α <sup>3.7</sup> /αα
11	4.06	10.5	31.3	76.9	25.8	33.6	HbA/S	-α <sup>3.7</sup> /αα
12	4.65	14.1	39.1	83.9	30.3	36.1	HbA/S	-α <sup>3.7</sup> /αα
13	4.73	12.5	34.5	73.0	26.4	36.2	HbA/S	-α <sup>3.7</sup> /αα
14	4.26	11.4	32.3	75.8	26.8	35.3	HbA/S	-α <sup>3.7</sup> /αα
15	4.43	9.4	29.7	67.1	21.3	31.7	HbA/S	-α <sup>3.7</sup> /αα
16	4.92	12.0	35.2	71.5	24.5	34.1	HbA/S	-α <sup>3.7</sup> /αα
17	5.30	9.7	29.8	55.2	18.0	32.6	HbA/S	-α <sup>3.7</sup> /-α <sup>3.7</sup>
18	5.50	9.7	29.3	53.2	17.6	33.1	HbA/S	-α <sup>3.7</sup> /-α <sup>3.7</sup>
19	4.46	7.3	25.0	56.0	16.4	29.3	HbA/S	-α <sup>3.7</sup> /-α <sup>3.7</sup>
20	5.65	10.1	33.1	58.5	18.0	30.7	HbA/S	-α <sup>3.7</sup> /-α <sup>3.7</sup>
21	5.23	15.2	42.7	81.5	29.1	35.7	HbA/S	-α <sup>3.7</sup> /-α <sup>3.7</sup>
22	4.79	8.7	26.2	54.5	18.1	33.1	HbA/S	-α <sup>3.7</sup> /-α <sup>3.7</sup>
23	5.10	8.9	27.0	52.8	17.5	33.0	HbA/S	-- <sup>MED</sup> /-α <sup>3.7</sup>
24	5.07	8.9	26.0	51.3	17.5	34.2	HbA/S	-- <sup>MED</sup> /-α <sup>3.7</sup>
25	4.59	11.4	33.8	73.6	24.8	33.7	HbA/S	ααα <sup>3.7</sup> /αα
26	2.23	6.5	19.7	88.1	29.1	32.9	HbS/S	-α <sup>3.7</sup> /αα
27	2.18	6.7	19.6	89.9	30.6	34.2	HbS/S	-α <sup>3.7</sup> /αα
28	4.90	13.0	35.7	72.8	26.5	36.4	HbS/S	-α <sup>3.7</sup> /-α <sup>3.7</sup>
29	4.83	8.6	28.9	59.7	17.7	29.6	HbS/S	-α <sup>3.7</sup> /-α <sup>3.7</sup>
30	2.56	7.6	21.4	83.3	29.7	35.5	HbS/S	-α <sup>3.7</sup> /-α <sup>3.7</sup>
31	2.56	8.5	26.4	103.3	33.4	32.3	HbS/S	ααα <sup>3.7</sup> /αα
32	2.38	7.4	19.9	83.4	31.2	37.4	HbS/S	ααα <sup>3.7</sup> /αα
33	3.72	10.0	30.4	81.7	27.0	33.0	HbS/D	α <sup>PolyA-2</sup> /α/αα
34	5.56	11.7	36.9	66.3	21.0	31.7	HbA/D	α <sup>PolyA-2</sup> /α/αα
35	4.31	9.8	28.7	65.7	22.7	34.5	IVS1.110/S	-α <sup>3.7</sup> /αα
36	5.18	11.8	35.4	68.4	22.8	33.3	IVS1.110/A	-α <sup>3.7</sup> /αα

Furthermore, two Hb S/S and one Hb A/S cases were detected to have concomitant anti-3.7 gene triplication. According to our data, co-existing anti-3.7 gene triplication with Hb A/S only slightly altered the hematological analyses. In Hb S/S, however, presence of anti-3.7 gene triplication did not affect the hematological presentation of the disease.

Higgs *et al.* studied the interaction of α-gene triplication with different β-globin genotypes including Hb A/A, A/S, A/C, S/S, and S/C. Consistent with our results, they found that the ααα/αα genotype had no effect on the clinical or hematological parameters of individuals with A/A, S/S, or S/C genotypes, but it was associated with a significantly

increased level of Hb S or Hb C in heterozygotes for these variants [21].

We have found that one of two subjects with  $\alpha^{\text{PolyA-2}}\alpha/\alpha\alpha$  mutation in his  $\alpha$ -globin gene had co-inherited Hb S/D and the other Hb A/D. As far as we know, no subjects with Hb S/D or Hb A/D who had concomitant  $\alpha^{\text{PolyA-2}}\alpha/\alpha\alpha$  mutation have been reported so far, based on the data gathered from the HbVar Database [22]. We observed that the  $\alpha^{\text{PolyA-2}}\alpha/\alpha\alpha$  mutation resulted in lower MCV, MCH and MCHC values in a Hb A/D case compared to a Hb S/D subject.

Uncomplicated sickle cell traits usually have a normal blood examination as assessed by conventional clinical methods, including normal red cell morphology, indices, reticulocyte counts, etc. Loss of one or two  $\alpha$ -globin genes, however, results in a decrease of the fraction of Hb S and produces an apparent microcytosis which is important from a genetic counseling perspective [23]. In our analysis, we detected 25 sickle cell traits with coexisting  $\alpha$ -globin gene abnormalities; most of them had hypochromic and/or microcytic anemia without iron deficiency. We were consequently able to provide the most accurate genetic counseling for these individuals.

Several studies have suggested that SCA patients with co-inherited  $\alpha$ -thal have significantly increased mean levels of Hb, HCT, red blood cell counts, HbA<sub>2</sub> and decreased MCH and MCV as compared to those with a normal genotype ( $\alpha\alpha/\alpha\alpha$ ) [24-27]. Moreover, it has also been observed in some of these studies that as the number of  $\alpha$ -globin genes decreases, an evident reduction in the MCV, MCH and MCHC values occurs [25-27]. In our study, we identified seven Hb S/S patients with coexisting  $\alpha$ -globin gene mutations. Despite the fact that we had a relatively low number of patients with concurrent SCA and  $\alpha$ -thal in our group, the hematological profile of this group was noted to be consistent with those reported in the above-mentioned studies. Namely, we obtained the lowest Hb and HCT results in two patients with  $\alpha^{3.7}$  single gene deletion ( $-\alpha^{3.7}/\alpha\alpha$ ), whereas the patients with  $-\alpha^{3.7}/-\alpha^{3.7}$  deletions had significantly higher values. Also, a noticeable decrease in MCV and MCH values was observed in patients with  $-\alpha^{3.7}/-\alpha^{3.7}$  deletion. Last of all, the presence of excess  $\alpha$  genes ( $\alpha\alpha\alpha^{3.7}/\alpha\alpha$ ) in SCA patients resulted in similar hematological data as seen for cases who had  $-\alpha^{3.7}/-\alpha^{3.7}$  mutation.

In compound heterozygotes for  $\beta$ -thal and Hb S (IVS1.110/S), the red cell indices were significantly different with concurrent  $\alpha$ -thal mutation ( $-\alpha^{3.7}/\alpha\alpha$ ) although the IVS1.110/A with  $-\alpha^{3.7}/\alpha\alpha$  genotype is associated with better hematologic status.

There is also increasing interest in the negative epistatic effect between  $\alpha$ -thal and the sickle cell

trait with respect to protection against malaria. Some recent studies have indicated that although  $\alpha$ -thal and sickle cell trait are independently associated with resistance to clinical malaria, their protective effect disappears when inherited together [28, 29]. Penman *et al.* argued that a possible explanation for this negative epistatic interaction might be the reduction of intra-erythrocytic Hb S concentration in such cases [28]. Although we did not investigate our group for malarial infections, studying individuals with complex hemoglobinopathies appears to be an important approach to understand the mechanisms of malaria protection in specific conditions.

In conclusion, given that interactions between the  $\alpha$ - and  $\beta$ -globin chains may produce moderate to severe phenotypes depending on the molecular defects involved, Hb analysis alone may not be accurate, especially in populations with a high frequency of different globin gene mutations. A study of the ameliorating effects of  $\alpha$ -thal on the red cell indices of patients with sickle cell trait would be useful for diagnosis of atypical carriers and valuable if different effects can be found with different  $\alpha$ -thal mutations. Nevertheless, red cell indices in our group showed no specific correlations with the  $\alpha$ - and  $\beta$ -globin gene status. In addition, MCV, MCH, and MCHC values were higher than those reported for this association in the literature.

In this paper, we intended to report our results of a study regarding the co-inheritance of  $\alpha$ - and  $\beta$ -globin gene deficiencies in Adana, Turkey. However, due to the low number of cases in this study, it was not possible to make a concise interpretation of the effect of coexisting  $\alpha$ -globin chain mutations on the phenotype of cases with  $\beta$ -globin chain abnormalities. Still, it seems to be useful to investigate  $\alpha$ -globin deletions in addition to  $\beta$ -globin gene mutations during the screening programs for premarital couples, especially in populations with a high frequency of hemoglobinopathies, and further studies with larger numbers of subjects are required to reach definitive conclusions.

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