



Codon 8 (-AA) and Codons 22/23/24 (-AAGTTGG) Compound Heterozygous Deletion Mutation in the β -Globin Gene: The First Report in Turkey

Cemal Polat^{1*}, Osman Evliyaoğlu², Nuriye Mete³, Esra Türk⁴, Ahmet Yıldırım⁵

¹Department of Biochemistry, Public Health Laboratory, Kutahya, Turkey

²Department of Biochemistry, Okmeydanı Education and Research Hospital, Istanbul, Turkey

³Department of Biochemistry, Faculty of Medicine, Dicle University, Diyarbakir, Turkey

⁴Faculty of Medicine, Harran University, Sanliurfa, Turkey

⁵Department of Pediatrics, Tekirdag State Hospital, Tekirdag, Turkey

Email: drcpolat@gmail.com

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Abstract

Background: Presentation of the first case of beta-thalassemia compound heterozygous with known mutations. **Case Report:** The patient was 3 years old girl. First symptoms were cough, fatigue, paleness. Hepatosplenomegaly were determined. Hematology parameters were: RBC 1.79 M/uL, Hb4.6 g/dL, Hct 12.3%, MCV 68.7fL, MCH 25.7pg, RDW 31.5%. The level of hemoglobin variants was: HbF 80.5%, HbA 18.2%, HbA2 1.3%, and by molecular analysis codon 8 (-AA) and codons 22/23/24(-AAGTTGG) compound heterozygous mutations were detected. Codon 8 (-AA) and codons 22/23/24(-AAGTTGG) heterozygous mutations were also detected in patient's mother and father respectively. **Conclusion:** Clinical manifestations such as late onset of symptoms and laboratory findings of patient with compound heterozygous deletion mutation were worse than homozygous patients whom are having the same mutations. It is important that the detection of carriers before the marriage to prevent the birth of patient children and genetic counseling is a good variety of ways of informing the public on the importance of prenatal diagnosis.

Keywords

Beta-Thalassemia, Compound Heterozygous Deletions, Codon 8 (-AA), Codons 22/23/24 (-AAGTTGG)

Subject Areas: Biochemistry, Genetics, Hematology, Pediatrics

*Corresponding author.

1. Introduction

Beta (β)-thalassemia is an inherited disorder characterized by the inability to synthesize sufficient β -globin chain which is controlled by the gene located on the short arm of chromosome 11. β -thalassemia is a single-gene disease and has autosomal recessive pattern, especially observed in the Mediterranean Region countries, characterized by hemolytic anemia and microcytosis. Patients with β -thalassemia require life-long blood transfusion and iron chelation therapy ensure enough hemoglobin (Hb) level to prevent the accumulation of iron. While the rate of β -thalassemia carriers is 2% in overall country, but in some regions of Turkey ratio can be increase up to 10%. In much of the population (in a geographic area or ethnic groups), more than 90% of cases have been found to be limited by mutation of 5 to 6, which are the most commonly detected mutations such as IVS I-110 (G \rightarrow A), IVS I-6 (T \rightarrow C), IVS II-745 (C \rightarrow G), IVS I-1 (G \rightarrow A), F508del (-AA), IVS II-1 (G \rightarrow A) [1]-[6].

In general, patients with β -thalassemia major have deletion mutations in a single gene, double-deletion mutation observed in our case. It was interesting observation the association of these mutations in Diyarbakır that was not found in the previous publications.

2. Case Presentation

A 3 years old girl admitted to our clinic in 05.02.2013 (Dicle University, Children Hospital, Department of Hematology), with complaint of cough, paleness, weakness. Hepatosplenomegaly were determined by clinical examination. Complete blood count parameters were (Cell Dyn 3700, Abbott Laboratories, Abbott Park, IL, USA) RBC 1.79 M/uL, Hb 4.6 g/dL, Hct 12.3% , MCV 68.7 fL, MCH 25.7 pg, RDW 31.5%. Patient was followed up with the diagnosis of anemia. By the method of High Performance Liquid Chromatography, (Agilent 1100 series, Agilent Technologies, Waldbronn, Germany) variants of Hb determined as HbF 80.5%, HbA 18.2%, HbA2 1.3%. In the light of the follow-up β -thalassemia major diagnosed. Analysis of β -globin gene mutations was performed from whole blood samples, DNA was extracted and isolated from two pairs of primary (GML, Switzerland) then PCR (polymerase chain reaction) was performed. Sequence analysis of the gene determined with Big Dye[®] Terminator v3.1 Cycle Sequencing kit (Applied Biosystems, Inc., Foster City, CA, USA) and ABI PRISM 310 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). β -globin mutations were detected with SeqScape Software v2.6 program. Codon 8 (-AA) and codons 22/23/24 (-AAGTTGG) compound heterozygous mutations were detected (**Figure 1**). Codon 8 (-AA) heterozygous deletion was found in mother's sample and codons 22/23/24 (-AAGTTGG) heterozygous deletion mutation was found in father's sample (**Figure 1**). Verbal and written consent was obtained from the family.

3. Discussion

Large parts of β -thalassemia mutations are point mutations. Deletions are lesser extent [7]. β -thalassemia minor is a mild form of the disease. Individuals are heterozygous for the mutant allele. More than 200 mutations affecting the β -globin gene are now known to result in a phenotype of β -thalassemia. More than 40 of these mutations were reported in Turkey [8]-[10].

Patient's mother's mutation were at codon 8 (-AA) and father's mutation were at codons 22/23/24 (-AAGTTGG). Both of them were heterozygous mutations and diagnosed clinically as β -thalassemia minor compatible with the hematologic parameters (**Table 1**). β -thalassemia major is observed in patients with homozygous or compound heterozygous with a mutant β -globin allele. Codon 8 (-AA) deletion mutation is often seen in Turkey, but codons 22/23/24 (-AAGTTGG) deletion mutation is rare [3] [11]. Codon 8 (-AA) and codons 22/23/24 (-AAGTTGG) compound heterozygous mutations have not been reported in Turkey yet. Homozygous form of these two deletions was published in the literature which was presented clinically as β -thalassemia major but there was no case presented as β -thalassemia major which had been having compound heterozygous of above mutations. Hematology parameters of homozygous and heterozygous mutations of codon 8 (-AA) and codons 22/23/24 (-AAGTTGG) are shown in **Table 2** [8] [12]. Codons 22/23/24 (-AAGTTGG) heterozygosity and Codon 8 (-AA) mutation heterozygosity showed mild presentation separately. In spite of the absence of HbA in homozygosity of Codon 8 (-AA) mutation, compound heterozygosity of these mutations has led to a more dramatic statement in Hb value.

β -thalassemia major is usually diagnosed during the first two years of birth [6]. Symptoms emerge during the second six months of life when gamma globin chain production decreases and is normally replaced with the

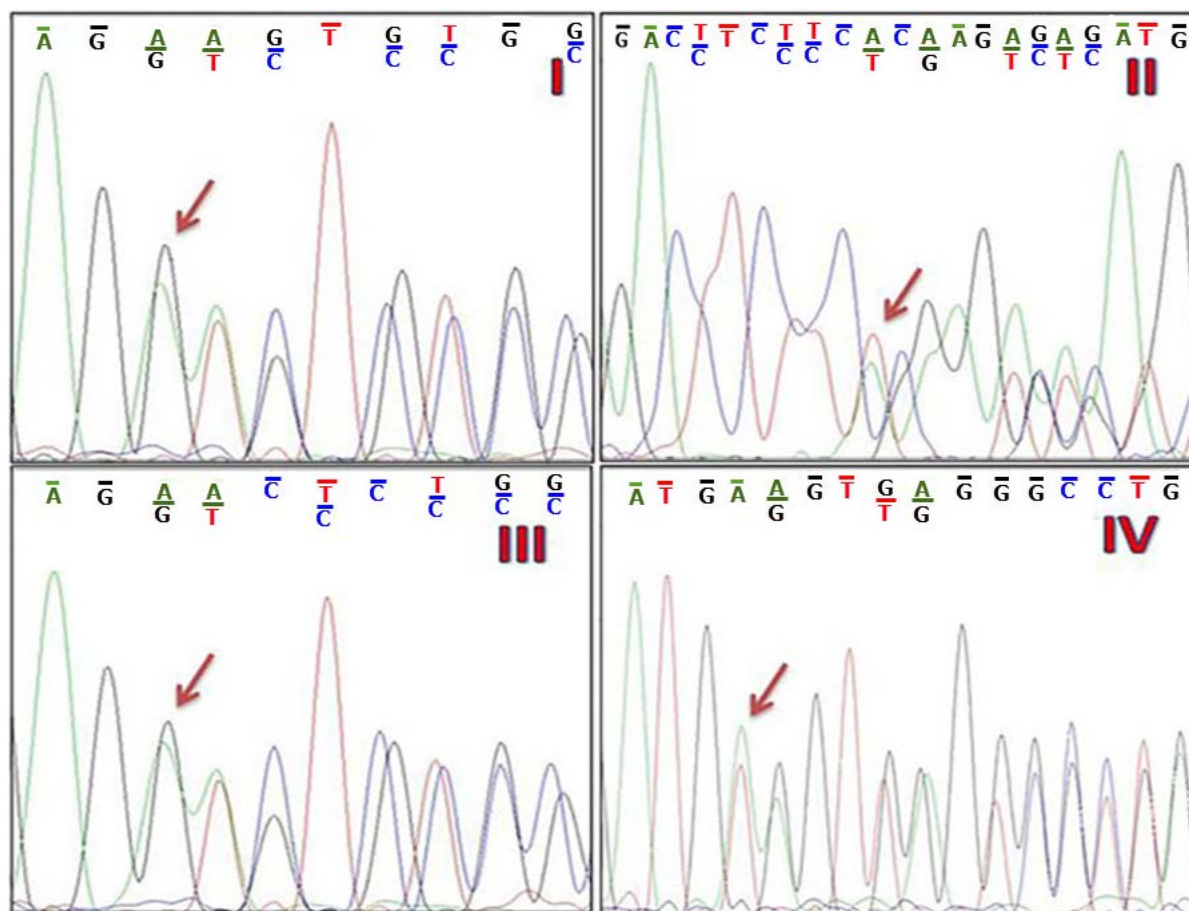


Figure 1. Deletions of β -globin genes. Patient [I \rightarrow codon 8 (-AA), II \rightarrow codons 22/23/24 (-AAGTTGG)], mother [III \rightarrow codon 8(-AA)] and father [IV \rightarrow codons22/23/24 (-AAGTTGG)].

Table 1. Hematologic parameters and hemoglobin variant levels of patient and parents.

	RBC (M/uL)	Hb (g/dL)	Hct (%)	MCV (fL)	MCH (pg)	RDW (%)	HbF (%)	HbA (%)	HbA2 (%)
Patient	1.79	4.6	12.3	68.7	25.7	31.5	96.25	1.84	1.90
Mother	5.31	9.6	29.9	56.4	18.2	19.9	2.11	92.57	5.31
Father	6.22	12.1	37.1	59.7	19.5	19.0	2.28	92.03	5.68

Table 2. Hematology parameters and values of Hb variants.

	Mutation Type	Hb (g/dL)	MCV (fL)	MCH (pg)	HbA2 (%)	HbF (%)	HbA (%)
Codon 8 del	Heterozygous [8]	9.7 - 12.8	68 - 74	16.3 - 18.8	4.1 - 5.1	0.7 - 3.8	91.1 - 95.2
	Homozygous [8]	10.6	82	29.4	1.4	98.6	0
Codons 22/23/24 del	Heterozygous [12]	10.3	64.8	24.9	5.4	3.1	92.5
	Homozygous						
Codon 8 and Codons 22/23/24 del	Compound Heterozygous	4.6	68.7	25.7	1.90	96.25	1.84

8 and 12 are the reference numbers.

production of beta globin to form adult hemoglobin (Hb A, α_2/β_2). The clinical expression of the severe phenotype is remarkably heterogeneous, depending upon a variety of factors that alter the burden of alpha-globin inclusions in the individual patient. When β -thalassemia major becomes clinically apparent during the second

six months of life, pallor, irritability, growth retardation, abdominal swelling due to hepatosplenomegaly, and jaundice reflect the onset and sequelae of severe hemolytic anemia. The symptoms associated with ineffective erythropoiesis (e.g., bony abnormalities and abnormal skeletal development) soon follow.

But clinically, this new compound heterozygous mutation became evident at three years old. This atypical presentation resulted in late diagnosis of the disease. It can be stated that compound heterozygous mutations impair the posttranscriptional or posttranslational regulation of hemoglobin chain more sophisticated manner than simple heterozygous mutations. The explanation of the delay in the clinic presentation of the disease needs more detailed investigations.

As consanguineous marriages in our country are very common and this culture increases the prevalence of the genetic diseases. In order to prevent the genetic diseases, it should be considered to detect the carriers before marriage, genetic counseling after marriage, informing the public on the importance of prenatal diagnosis.

Conflict of Interest

The authors declare no conflict of interest.

Financial Disclosure

In our study were excluded from the financial support.

Poster Presentation

Association of Clinical Biochemistry Professionals, International Participation Congress & Lab Expo, 24-28 September 2013, Antalya.

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